# PHYTOREMEDIATION POTENTIAL OF SWEET SORGHUM IN MERCURY-CONTAMINATED SOIL

by

**Idris Oladimeji Dauda**

Submitted in accordance with the requirements

for the degree

**Master of Science in Environmental Science**

in the

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

DEPARTMENT OF ENVIRONMENTAL SCIENCES

at the

**UNIVERSITY OF SOUTH AFRICA**

FLORIDA CAMPUS

**SUPERVISOR**

Mrs TC Fouche

**October 2020**

# DEDICATION

This project is dedicated to Almighty Allah: Glory is to Allah and praise is to Him, by the multitude of His creation, by His Pleasure, by the weight of His Throne, and by the extent of His words.

UNISA college of agriculture and environmental sciences

# DECLARATION

I, **Idris Oladimeji Dauda** hereby declare that the dissertation, which I hereby submit for the degree of **Master of Science** at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other institution.

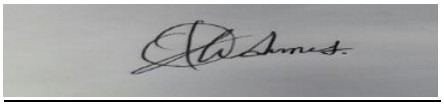
I declare that the dissertation does not contain any written work presented by other persons whether written, pictures, graphs or data or any other information without acknowledging the source.

I declare that where words from a written source have been used the words have been paraphrased and referenced and where exact words from a source have been used the words have been placed inside quotation marks and referenced.

I declare that I have not copied and pasted any information from the Internet, without specifically acknowledging the source and have inserted appropriate references to these sources in the reference section of the dissertation or thesis.

I declare that during my study I adhered to the Research Ethics Policy of the University of South Africa, received ethics approval for the duration of my study prior to the commencement of data gathering, and have not acted outside the approval conditions.

I declare that the content of my dissertation/thesis has been submitted through an electronic plagiarism detection program before the final submission for examination.

Student signature:  Date: **23/10/2020**

## ABSTRACTS

The continuity of the menace of mercury (Hg) is due to the continuous production and use of Hg and Hg containing products. Toxicity is just an outfall of use and exposure. Anthropogenic activities such as coal combustion and artisanal and small-scale gold mining have led to increasing Hg contamination and is the major source of Hg pollution into the environment that needs to be remediated. This study aimed to assess the phytoextraction capability of sweet sorghum (*Sorghum bicolor*) under different fertiliser treatments in Hg-contaminated soil. The potted experiment in a controlled environment included control *S. bicolor* and three phytoremediation treatments, i.e., Hg only; the addition of 4:1 green compost and; the addition of 0.2% NPK fertiliser. There were conspicuous signs of Hg phytotoxicity in plants with Hg only, namely wilting, senescent, inhibition of growth, and photosynthesis. There was stunted growth, but healthy plants observed in the treatment with the addition of green compost towards the end (day 60) of exposure. However, *S. bicolor* grew well until the last day of exposure in the treatment with the addition of 0.2% NPK fertiliser. Thus, this treatment showed the most effective phytoextraction potential of *S. bicolor* in Hg-contaminated soil. The effectiveness of *S. bicolor* in reducing the level of mercury was best assessed in the Hg bioavailable concentration in the spiked soil in which the Hg + NPK treatment has the lowest (0.77 mg $kg^{-1}$). That resulted in the highest uptake (84.31%) percentage of Hg concentration recorded in the treatment with the addition of 0.2% NPK fertiliser compared to the other two treatments. The results suggest that the proportion of phosphate in the NPK fertiliser used, plays a huge role in the phytoextraction of Hg in the contaminated soil by *S. bicolor*. The Translocation Factor (TF) and Bioconcentration Factor (BCF), although higher within Days 20 and 40, was greater than 1 at the end of the exposure period suggesting a high probability that Hg was significantly transferred to the aerial parts of the plants. This is regarded as typical hyperaccumulator plant species. While *S. bicolor* was able to reduce the level of Hg in all three treatments, Hg + NPK treatment gave overall best results in physiological growth, the uptake, and reducing the level of Hg bioavailable in the spiked soil in terms of the effectiveness of phytoremediation method.

**ACKNOWLEDGEMENT**

All praises due to Allah (*Subhanahu wa ta'ala*) for His infinite and inexplicable mercy showers blessing, mercy upon me, protect, and guide me through from the beginning of this project till the very end. I would not have come this far without Him – *Alhamdulillah Rabbil Alamin.*

I gratefully acknowledge the financial support of the University of South Africa Experimental Masters and Doctoral Bursary made this work and the entire program possible. My profound and sincere gratitude goes to my able supervisor, Mrs. Tanya Fouche for her guidance, perpetual moral and financial support, encouragement, and constructive comments and corrections throughout the course of this research work. That greatly improved the quality of this project and shaping me to be a better researcher. Your efforts and kindness are reckoned and highly appreciated.

Extensive thanks go to Prof. VM Ngole-Jeme in the Department of Environmental Sciences, Florida Campus, who constructively previewed my research proposal and whose all her comments and feedbacks made the project a lot easier for me. Sincere appreciation goes to all the staff in the Horticulture Centre who accommodated me for a whole year of my experiment in the greenhouse. I am indebted to a fellow master student of the Chemistry Department, Jabu, who guided me using microwave digestion in the lab and all the staff at Botany, Biochemistry, and Environmental Sciences Labs for their support. Most importantly, Ms. Mpho Kotoane for accommodating me in her laboratory and shared a lab with her from the beginning until the end of the research project. She was also very helpful in analysing all the plant and soil samples with ICP-OES in her laboratory.

I like to thank Mr. Rotimi Sanyaolu for his tutor and guidance on statistical analysis. Appreciation goes to my "friend like a Brother" Engineer Ibrahim Salako for transporting all my research materials and equipment to the Florida Campus and hosting me all the time at his place in Pretoria throughout the course of this program. So, I say *Jazakallah* to you all!

Finally, I am very grateful to my Mom, sister (Mrs. Bushirat Temitope Abdullateef), and brothers (Taofeek and Muideen Dauda) for their unending love, and support given to me over the years.

**Idris Oladimeji DAUDA**

# TABLE OF CONTENTS

**CONTENTS** **PAGE**

**LIST OF TABLES** **PAGE**

**LIST OF FIGURES** **PAGE**

## DEFINITIONS OF KEY TERMS, CONCEPTS AND VARIABLES

The definitions of key terms and concepts used in the study are briefly defined below as thus:

- Phytoremediation: This refers to the use of green plants and their associated microbes for environmental clean-up.
- Mercury (Hg): refers to the contaminant in this context that was remediated. It is a naturally occurring heavy metal that is found in air, water and soil.
- Contaminated soil: refers to the potting soil that was used in the study which was spiked with inorganic mercury.
- Sweet sorghum: Also refers to as *Sorghum bicolor*, the plant that was used for phytoremediation process to extract mercury from the spiked soil.
- Organic fertiliser: refers to the green compost that was used as one of the treatments for the phytoremediation study to evaluate its effect on phytoextraction capability of sweet sorghum.
- Inorganic fertiliser: refers to nitrogen-phosphate-potassium (NPK) fertiliser that was used as one of the treatments for the study to evaluate its effect on the phytoextraction capability of sweet sorghum.
- Phytoextraction: refers to the uptake of a contaminant (mercury) from the soil (spiked) by plant (sweet sorghum) roots and their translocation to and accumulation in aboveground biomass.
- Translocation factor (TF): refers to the ratio of Hg concentration in the shoots to the roots.
- Bioconcentration factor (BCF): refers to the ratio of the Hg concentration in the roots to that in the soil.
- Bioavailable: Fraction of Hg concentrations that is available in the soil substrate after the last exposure period of sweet sorghum to mercury in Hg-contaminated soil.
- Heavy metals: refers to naturally occurring elements that has a relatively high density and is toxic or poison at very low concentrations. They are also known to be almost indefinite persistence in the environment.
- Hyperaccumulators: refers to the plants that are capable of accumulating heavy metals in their aboveground tissues far exceeding levels present in the soil.

# CHAPTER 1

## 1. INTRODUCTION

Globally, there is an increasing trend in areas of land degradation, especially agricultural soils, surface waters, and underground water affected by contamination from industrial, military, and agricultural activities (USEPA 2008; Sarwar *et al.,* 2017). Soil is the basic environmental element constituting a major component of the terrestrial ecosystem and equally important material basis for food production. However, the pollution of soil is a major concern (Hidayati *et al.*, 2009). According to the Agency for Toxic Substances and Diseases Registry (ATSDR, 2009), contamination of soil and water with heavy metals such as arsenic, lead, mercury, chromium, zinc, cadmium, uranium, selenium, silver, gold, and nickel is a major environmental concern and a great threat to life on earth. Mercury has been considered as one of the top ten toxic chemicals that are of major public health concern (WHO, 2019). There are many health risks associated with heavy metals contamination especially when they enter the food chain (Ashraf *et al.,* 2013; Sarwar *et al.,* 2017). Given this, the build-up of these toxic pollutants not only affects natural resources but also causes a greater strain on the ecosystem (Ashraf *et al.,* 2013).

Mercury (Hg) is a naturally occurring metal found in air, water, and soil. It exists in different forms, such as elemental or metallic mercury, inorganic mercury compounds, and organic mercury compounds, but each with distinct properties, usage, and toxicity (ATSDR, 2009; Hidayati *et al.,* 2009; Kumar *et al.,* 2014). The most common mercury in the environment is methylmercury, which is produced mainly by microbes, such as bacteria, in soils and water, however higher concentrations of mercury in the environment are due to anthropogenic activities namely artisanal gold mining (ASGM) and industrial usage in automobile, electronics, dyes, cosmetics, paints, pharmaceuticals and chemical processes (NCBI, 2018). These activities can increase the amounts of methylmercury that is being produced by bacteria. It has been reported that eating fish or shellfish is usually how methylmercury is ingested (Kumar *et al.,* 2014). Exposure to high levels of metallic, inorganic, or organic mercury have been reported to cause damage to the immune, digestive and nervous systems, as well as kidneys, developing foetuses, and permanent brain damage. Short term exposure to high levels of metallic mercury vapours may also cause severe effects, such as lung damage, nausea, vomiting, diarrhoea, blood pressure or heart rate increases,

eye irritation, and skin rashes (ATSDR, 2009; Hidayati *et al.*, 2009; Kumar *et al.*, 2014; WHO, 2019). Therefore, due to the high persistence of mercury in the environment and the high risk to environmental and human health, remediation of contaminated sites is necessary.

Remediation of contaminated sites is usually done by employing physicochemical methods, such as chemical precipitation, electrolytic recovery, ion exchange/chelation, solvent extraction/liquid membrane separation, and size exclusion pressure (Esalah *et al.*, 2000; Li *et al.*, 2009; Shao *et al.*, 2010).

These approaches to remediation have limited potential and are usually only applicable to small areas, and often make the soil infertile and unsuitable for agriculture and other uses by destroying the microenvironment (Shao *et al.*, 2010; Ashraf *et al.*, 2013; Titah *et al.*, 2013; Kumar *et al.*, 2014). Therefore, new technologies are required to remove and/or reduce these contaminants to environmentally acceptable levels at affordable costs. Phytoremediation with low-cost materials (industrial, agricultural, or urban residues) has emerged as a promising technology for recovering mercury and lead from contaminated sites (Ashraf *et al.*, 2013; Kumar *et al.*, 2014). Phytoremediation is the use of green plants and their associated microbes for environmental clean-up (Pilon, 2000; USEPA, 2008; Ashraf *et al.*, 2013; Titah *et al.*, 2013). Phytoremediation has been regarded as an increasingly recognised pathway for contaminants removal from water and shallow soils and described as aesthetically pleasing, solar-driven and it is useful for remediation of heavy metals (Ashraf *et al.*, 2013). Phytoremediation is an environmentally friendly, safe, and cheap technique to eliminate the pollutants. Phytoremediation of toxic metals from the contaminated soil involves the extraction or inactivation of these metals in soils (Pilon, 2000; Xia *et al.*, 2003; Ashraf *et al.*, 2013; Titah *et al.*, 2013; Kumar *et al.*, 2014).

## 1.1 The research problems

The increasing levels of mercury species in the environment, their entry into the food chain, and overall health effects are of great concern to researchers in the field of environmental science. This is not only because of the great threat heavy metals pose to life on earth but also due to their almost indefinite persistence in the environment (Kumar *et al.*, 2014; Natasha *et al.*, 2020).

The toxicity of mercury in soil and water will continue as a menace globally if there is an increase in its entry concentration in soil, water, and air due to natural and anthropogenic activities. It has been reported that artisanal and small-scale gold mining (ASGM) is the largest sector of demand for mercury, and the largest source of mercury pollution to air, soil, and water bodies (UNEP, 2013). This results in one-third of mercury emitted, to end up in piles of mining waste (e.g., tailing), water bodies, and soils (Telmer and Stapper, 2012). This is due to ignorance, lack of vision, or carelessness and/or because of the enormous cost that is associated with remediation of contaminated sites using conventional practices or physicochemical techniques (Li *et al.*, 2009; Shao *et al.*, 2010; Ashraf *et al.*, 2013).

The conventional technologies to remediate Hg contaminated media include 'pump and treat' and 'dig and dump' techniques, *in situ* thermal desorption; electro-kinetics; and soil flushing/washing (Wang *et al.*, 2012; He *et al.*, 2015). These are known mobilisation removal technologies. However, these are all associated with the very high cost and this has been regarded to often encourage the companies responsible to ignore the problem (Ashraf *et al.*, 2013; Kumar *et al.*, 2014). Conventional technologies are also argued to only apply to selective soils, have potential risks for the release of Hg vapour, and have negative impacts on the ecological health at the treated sites (USEPA, 2007). *In situ* soil flushing/washing, to be specific has been declared unacceptable to regulators and public and intrusive to the treated area (Truex *et al.*, 2010; He *et al.*, 2015). Known immobilisation technologies are solidification and vitrification. Solidification have been reported to introduce chemical agents that may cause problems themselves and increase the volume of the contaminated area, rather than remediating it. This poses risks in a long term because of that. Vitrification, on the same hand, is very energy-intensive and requires the necessary capture and treatment of given off-gases during the vitrification method at the contaminated site (Dermont *et al*., 2008).

## 1.2 The research questions

The research questions for this study are as follow:

- What enable and classify sweet sorghum to be used as hyperaccumulator plant species for phytoremediation with its phytoextraction capability?
- What are the potentials of sweet sorghum to reduce the level of mercury in the contaminated soil over 60 days of exposure?
- Which type of fertiliser (organic or inorganic) will be most effective on the phytoremediation capability of sweet sorghum in Hg-contaminated soil?

## 1.3 Aim of the study

The aim of this study is to assess the phytoextraction capability of sweet sorghum (*S. bicolor*) under different fertiliser treatments in mercury-contaminated soil. In this study, the effects of organic and inorganic fertiliser treatments on inorganic mercury uptake and accumulation by *S. bicolor* during phytoremediation in a controlled environment (greenhouse), was investigated.

## 1.4 The objectives of the study

The objectives of this study are as follows:

- to determine if sweet sorghum can be classified as hyperaccumulator plant species for Hg contaminated soil in a controlled environment;
- to assess to which extent sweet sorghum can reduce the level of mercury in the contaminated soil and;
- to examine if the treatment with organic (compost) and inorganic fertilisers (NPK) has any effect on the phytoextraction potential of sweet sorghum in mercury-contaminated soil.

## 1.5 Significance of the study

In previous studies, sweet sorghum has been shown to be a successful "hyperaccumulator" plant species for some heavy metals namely, Mn and Cd (Luo *et al*., 2012) Arsenic (Sathya *et al*., 2016), and Mercury (Kokyo *et al*., 2015). In the remediation of mercury particularly, sweet sorghum has not always been solely used to phytoremediate the contaminated soils, but in conjunction with a rhizo-bacteria consortium and nitrogen phosphorus potassium (NPK) fertiliser. Further, these were remediated with low mercury concentrations (0.890-6.755 µg $g^{-1}$) in correlation with Hg concentration in gold tailing in a controlled environment at Witwatersrand basin, South Africa (Malehase *et al*., 2016) and likewise in a field study at a small-scale artisanal gold mining region of Indonesia (Kokyo *et al*., 2015) with the mercury concentration of 3.8 mg $kg^{-1}$.

Conversely, in this study, sweet sorghum was assessed for its phytoextraction capability in a controlled (greenhouse) environment with monitored natural attenuation and enhanced application of organic (compost) and inorganic (NPK) fertilisers to compare their effects in reducing the mercury concentrations (13.54 mg $kg^{-1}$) in soils. According to Marrugo-Negrete *et al*. (2015) mercury is one of the most widespread pollutants that are derived from both natural and anthropogenic sources. The authors argued that mercury has a vast global impact due to its toxicity, complex chemo-dynamics in the environment, and the tendency to bio-magnify in the ecosystem. Therefore, there is a need to remediate the mercury contaminated environment. However, the chemical, physical or biological treatments are often expensive and affect soil properties, which may eventually render the soil unsuitable for agriculture as a medium for plant growth.

The potential benefits and how this study can benefit society are that, sweet sorghum can directly be grown on the contaminated land by the host communities of artisanal and small-scale gold mining to assist with. Sweet sorghum has the capability to absorb Hg and other contaminants in the soils, translocate the contaminants to the aerial parts and sequester mercury ions in the tissue of the plant during the period of growing on the contaminated soil. This not only make this method safe, cost-effective, and an easy biological remediation strategy but sweet sorghum can also be planted as a biofuel for ethanol production as another source of income. This could ease the stress and problem associated with limited fertile farmland between the energy crops and food crops (Luo *et al*., 2012; Sathya *et al*., 2016). Hence, phytoremediation methods have been reported to

help preserve the natural physical and biological properties of soil by reducing or removing the level of contaminants in the soil.

## 1.6 General outline

The content of this dissertation is organised into five chapters that are presented as thus:

- Chapter 1 includes an introduction to this study; the research problems, the research questions; aim of the study; the objectives of the study; the significance of the study; and the general outline of the study.
- Chapter 2 is a review of the chemistry of mercury, effects of mercury exposure, the origin of mercury in soil, fate, and transport of mercury in soil, remediation of mercury-contaminated soils: solidification/stabilisation; immobilisation; vitrification; thermal desorption; nanotechnology; soil washing; electro-remediation; phytoremediation; and sweet sorghum as phytoremediator.
- Chapter 3 entails research methods and materials employed in the experiments.
- Chapter 4 contains the results obtained in this research study and the discussion.
- Chapter 5 presents conclusion and recommendations.

# CHAPTER 2

# 2. LITERATURE REVIEW

## 2.1 Chemistry of mercury

Mercury (Hg) is a naturally occurring metal that is found in air, water, and soil. It exists in three forms, but with different properties, usage, and toxicity. They are elemental or metallic mercury, inorganic mercury compounds, and organic mercury compounds (ATSDR, 2009; Hidayati *et al*., 2009; Kumar *et al*., 2014). Mercury can combine with other elements, such as chlorine, sulphur, or oxygen, to form inorganic mercury compounds or "salts," which are white powders or crystals in nature. Mercury can combine with carbon to make organic mercury compounds. The physicochemical properties of mercury and its compound are outlined in Table 2.1.

**Table 2.1:** The physicochemical properties of mercury and its compounds (Schroeder *et al*.,1998)

| Properties | $Hg^0$ | $HgCl_2$ | HgO | HgS | $CH_3HgOH$ |
|---|---|---|---|---|---|
| Melting point (◦C) | -38.8 | 277 | 500 (decomposition) | 584 (sublimation) | 137 |
| Water-solubility ($g\ L^{-1}$) | $49.6\times10^{-6}$ (20 ◦C) | 66 (20 ◦C) | 0.053 (25 ◦C) | $2\times10^{-24}$ (25 ◦C) | - |
| Boiling point (◦C) | 356.7 | 303 | - | - | - |
| Vapor tension (Pa) | 0.18 | 0.009 | $9.2\times10^{-12}$ | nd | 0.9 |

The most common mercury is methylmercury which is produced mainly by microbes, such as bacteria, in soils and water. The higher concentrations of mercury in the environment due to anthropogenic activities can increase the amounts of methylmercury in the environment. It was reported that the artisanal and small-scale gold mining (ASGM) is the largest sector of demand for mercury, and the largest source of mercury pollution to air, soil, and water bodies (UNEP, 2013). This results in one-third of mercury emitted, to end up in piles of mining waste (e.g., tailing), water bodies, and soils (Telmer and Stapper, 2012). The enormous cost that is associated with

remediation of contaminated sites by conventional physicochemical techniques, the ignorance of people, the lack of vision has also contributed to the increased levels of mercury in our soils (Esalah *et al.*, 2000; Li *et al.*, 2009; Shao *et al.*, 2010; Ashraf *et al.*, 2013).

## 2.2 Effects of mercury exposure

Exposure to high levels of elemental, inorganic, or organic Hg has been reported to cause damage to kidneys, developing foetuses, and permanent brain damage. Short term exposure to high levels of metallic mercury vapours may also cause severe effects, such as lung damage, nausea, vomiting, diarrhoea, blood pressure or heart rate increases, eye irritation, and skin rashes (ATSDR, 2009; Hidayati *et al.*, 2009; Kumar *et al.*, 2014). The central nervous system (CNS) is also sensitive to mercury exposure, especially to methylmercury, and elemental Hg vapours are said to be more harmful than any other form of Hg because the brain is the site of action for these forms of mercury. Alkylated Hg (methylated Hg) is the most toxic. Elemental Hg is poorly absorbed with a bioavailability of less than 0.1% upon ingestion. It is lipid soluble, hence it easily crosses the blood-brain barrier making easy to induce toxic effect on the CNS. Additionally, the World Health Organization (WHO) stated that human exposure to Hg has been associated with numerous toxic effects on the immune, digestive and nervous systems, likewise on kidneys, lungs, eyes, and skin. Hence WHO considers Hg as one of the top ten toxic chemicals of major public health concern (WHO, 2019).

The brain and CNS susceptibility to mercury exposure were more evident with the study conducted by Feng *et al.* (2007) in which the result indicated that the significant brain damage in rats fed mercury-contaminated rice from the Wanshan Hg mining area than that from a control site. This was also supported by the study of Cheng *et al.* (2006) who suggested that rice with an elevated concentration of methylmercury was the main route of mercury exposure.

Mercury contamination has been regarded, globally, as one of the major environmental concerns not only because of its increased level in the environment but also due to its ability to be readily taken up by the plants and aquatic life and be biomagnified in the human body through the food

chain (He *et al*., 2015). In view of that, it is imperative for the development of methods to remediate the mercury-contaminated media.

## 2.3 Origin of mercury in soil

The origin of mercury contamination in soils occurs through natural events such as weathering of mercury-containing rocks, volcanic eruptions, and geothermal activity (Han *et al*., 2008; Wang *et al.*, 2012), or through forest fires (Ermolin *et al*., 2018; Natasha *et al*., 2020), which are known as natural sources. The natural geological sources of mercury are reported to account for about 10% of annual mercury emission (Mason *et al*., 1994; UNEP, 2013). On the other hand, global anthropogenic mercury emissions include emissions released from fuels, raw materials, or uses in products or industrial processes such as automobile production, electronics, dyes, cosmetics, paints, pharmaceuticals, plastic and chemical industries (NCBI, 2018). In addition, the emission of mercury from the refining of non-ferrous metals represents another major source of anthropogenic mercury which leads to the deposition of atmospheric mercury onto soils surrounding metal smelters. Other large sources of anthropogenic origin mercury are artisanal gold mining, coal combustion, military installations, wood/forestry impregnation sites, landfills, and waste incineration (Han *et al.*, 2008, Mason *et al.*, 1994, UNEP, 2013). About 30% of these are said to contribute to annual emissions of mercury (UNEP, 2013). The remaining 60% reported originating from the re-emissions of mercury that was once released and has built up over a long time in surface soils and oceans (Mason *et al*., 1994; Dastoor and Larocque, 2004; Wang *et al*., 2012; UNEP, 2013; Malehase *et al*., 2016).

According to the Agency for Toxic Substances and Diseases Registry (ATSDR, 2009), contamination of soil and water with heavy metals such as arsenic, lead, mercury, chromium, zinc, cadmium, uranium, selenium, silver, gold, and nickel, is a common problem encountered at many hazardous waste sites and mining dumping sites. These are regarded as a major environmental concern and a great threat to life, as there are many health risks associated with heavy metals regarding their entry into the food chain (ATSDR, 2009; Ashraf *et al.*, 2013; Sarwar *et al.*, 2017).

## 2.4 Fate and transport of mercury in soil

According to Walker *et al.* (2014), there are four major factors that can control the fate of inorganic pollutants in contaminated ecosystems, namely: localisation, persistence, bioconcentration, and bioaccumulation factors, and bioavailability. These are said to be influenced by the soil conditions such as pH, temperature, and soil moisture content (USEPA, 1997a).

- **Localisation:** When the concentration of a pollutant exceeds a threshold value (>10 mg $l^{-1}$) in an environmental compartment, such pollutant is said to be toxic (USEPA, 1997b; Walker *et al.*, 2014). The Hg concentrations determined at the gold mine tailing dam at Witwatersrand basin, South Africa (Malehase *et al.*, 2016); Phichil Province Thailand (Pataranawat *et al*., 2007), and Tongguan gold mining area in China (Feng *et al.*, 2006) all exceed environmental unsafe level (>10 mg $l^{-1}$).
- **Persistence:** Metals are nonbiodegradable and persistent in the environment for a long time. Therefore, Hg polluted soil remediation is particularly important. Wang *et al*. (2012) refers to the study by Semu *et al.* (1987) that found out that dry and wet mercury deposition can be trapped by organic matter and thereafter become enriched in the surface layers of soil. The distribution of total mercury in soil and the transport of mercury through soil profiles has been argued to be majorly influenced by the amount and the quality of organic matter (Lehmann and Kleber, 2015), and the mechanisms regulating the partition of organic matter between aqueous and solid phases. Soil bacteria are possibly able to transform the deposited Hg to a more toxic form of mercury known as methylmercury (Ullrich *et al.*, 2001).
- **Bioconcentration and Bioaccumulation Factors:** According to Walker *et al.* (2014) some inorganic pollutants are assimilated by organisms to a much greater extent than others. This is expressed as the bioconcentration factor (BCF):

$$BCF = \frac{\text{Concentration of chemical in the organism}}{\text{Concentration in the ambient environment}}$$

From an ecotoxicological point of view, Wolfe *et al.* (1998) argued that the extent of long-term bioaccumulation of inorganic chemicals depends on the rate of excretion. Hence bioaccumulation factor (BAF) is designed not only to predict chemical uptake through direct contact with or uptake from an environmental medium but also to account for any food chain

pathways that may in some manner connect the environmental medium to the biological medium of interest (USEPA, 1997b; Natasha *et al*., 2020). An organisms' biochemistry may cause it to exhibit a high BCF for a specific substance, Thus, BCF is one of the factors this study employed to determine the phytoremediation potential of sweet sorghum in mercury-contaminated soil.

- **Bioavailability:** This refers to the fraction of free metal ions that are available in the soil substrate for uptake by the biological component in the soil (plants and other organisms). A substance with a high BCF is generally more bioavailable than a substance with a low BCF. (Walker *et al*., 2014). For instance, unmethylated mercury is less readily available than methylated mercury (Wolfe *et al*., 1998). Further, pH has also been argued, as mentioned in this section, to influence the solubilities of metals in soils and water. This is because pH below 4.5 is said to increase the solubility of methylmercury (Wolfe *et al*., 1998; Walker *et al*., 2014) and this is readily available to be ingested by the aquatic lives which in turn enter the food chain (USEPA, 1997a; Natasha *et al*., 2020).

## 2.5 Remediation of mercury contaminated soils

Considering the growing need to address the environmental contamination of mercury and the potential health hazards that it poses to humans and the environment, there is a need to remediate the media contaminated by mercury. Many efforts by government, industry, and public have been undertaken to develop remediation methods and technologies to manage and reduce Hg contamination in soils. Both field and laboratory research have been undertaken. Remediation of mercury contaminated sites has been reviewed extensively by Wang *et al.* (2012) and most recently a critical review has been done by Natasha *et al*. (2020). Thus, various forms of treatment technologies have been employed in the remediation of mercury contaminated soils such as solidification/stabilisation, immobilisation, vitrification, thermal desorption, nanotechnology, soil washing, electro-remediation, phytoremediation (phyto-stabilisation, phyto-extraction, phyto-volatilisation, phyto-degradation) (Wang *et al.*, 2012).

### 2.5.1 Solidification/Stabilisation

This method is known to decrease the mobility of the hazardous contaminants in soils (He *et al.,* 2015). Solidification and stabilisation (S/S) methods involve physical binding or enclosing contaminants in the soil to reduce the waste surface and permeability of the contaminant. Wang *et al.* (2012) mentioned that cement or other binders such as bitumen, fly ash and gypsum, can be used to create a paste or other semi-liquid state which then allows it to cure or solidify. This helps to minimise the distribution and exposure of the contaminant (Trüe *et al.,* 2012; He *et al.*, 2015). This method is reported to be generally applicable to remove mercury or reduce Hg concentration in waste that is less than 260 mg $kg^{-1}$. This method is regarded as being effective if the final waste form leach is $\leq$ 0.2 mg $l^{-1}$ of mercury as assessed by the Toxicity Characteristics Leaching Procedure (TCLP) of the Resource Conservation and Recovery Act (RCRA). This has been reported as the required stabilisation method that must be achieved (He *et al.*, 2015; Wang *et al.*, 2012; Conley *et al*., 1999).

There are a few reported successful applications of this method, for instance, the study by Randall and Chattopadhyay (2010). Their study evaluated the stabilisation of mercury and mercuric chloride-containing surrogate test materials using chemically bonded phosphate ceramics (CBPC). The satisfactory requirement of <0.2 mg $l^{-1}$ was achieved for leachates from stabilised wastes containing <50 wt. % loading of elemental Hg and $HgCl_2$. In addition to that, S/S is also of advantages in immobilising contaminants and is reported to be acceptable by the US regulating agencies, the United States Environmental Protection Agency (USEPA). However, S/S has also been reported to increase the volume of the contaminated area, pose a risk over the long term, and ultimately introduce chemicals that can be a problem themselves to the supposedly remediated area (USEPA 1997b; USEPA, 2007). Thus, the significant increase in the waste's mass and volume over the long term and the need for future monitoring of the heavy metals remediated on site. There is a questionable longevity of the solidified/stabilised materials from the contaminated media. This has been argued as the backwardness of this remediating method (Wang *et al.,* 2012).

### 2.5.2 Immobilisation

According to He *et al.* (2015), immobilisation is an *in-situ* technology that does not only render the potential toxicity of mercury-contaminated soil immobile, but rather isolate, solidify, and stabilise the contaminant to reduce the ecological and harm risks from the exposure. This is achieved with the addition of stabilising ligands (sulphur-containing ligands, reducing agents, absorbing agents) to contaminated waste or soil (Wang *et al.*, 2012).

The use of sulphide to treat mercury-containing wastes with a mercury concentration as high as 2 300 mg $kg^{-1}$ was reported to be potentially less harmful because it is relatively insoluble and less volatile than other forms of mercury (Piao and Bishop, 2006). A reduction from 1 900 µg $l^{-1}$ for the untreated waste to 35 µg $l^{-1}$ was shown with the TCLP for Hg. Likewise, the addition of colloidal sulphur was argued to decrease the mercury concentration in soil solution significantly, as well as mercury accumulation by Kot *et al.* (2007).

The advantages of immobilisation are that it can be applied with other technologies to enhance Hg remediation effectiveness; low cost hence does not require excavation, and disposal of hazardous waste. However, the contaminants are reported to be left as is on the site (He *et al.*, 2015). The contaminant sites require long term sampling and monitoring of the stability of the resulting stabilised waste product (Wang *et al.*, 2012; He *et al.*,2015; USEPA, 1997); and above all, increase in the volume of waste (Wang *et al.*, 2012).

### 2.5.3 Vitrification

This is the method in which the contaminated soils and/or wastes are heated to the melting point and cooled to form a solidified and/or vitrified end product in which the contaminants are immobilised during the *in-situ* vitrification processes (USEPA, 2007; Wang *et al.*, 2012; He *et al.*, 2015). It was reported that the contaminant concentration reduces in the soil and waste as a result of volatilisation of the contaminants (He *et al.*, 2015). Also, Wang *et al.* (2012) mentioned that the vitrification technique is mainly used to remediate soil contamination with heavy metals mixed with the radioactive element of military installations.

The effectiveness of vitrification may be affected by soil's clay moisture contents, in correlation with thermal desorption treatment, however, *in situ* technique has been reported to be employed to treat large volumes of shallow soils (6-20 ft) (USEPA, 1997b; Mulligan *et al.*, 2001). Moreover, USEPA (2007) mentioned that vitrification does not also require excavation and off-site treatment and could be applied to a mixture of contaminants, and thus decreases the contaminant volume at the site due to the removal of organic material as one of the processes in which vitrification technique works.

The vitrification technique is said to be limited to the low concentration of mercury-contaminated soils associated with the high cost and the necessary treatment of off-gases, above all energy-intensive (USEPA 2007; Dermont *et al.*, 2008; Wang *et al.*, 2012).

### 2.5.4 Thermal desorption

This is a treatment technique in which the contaminants are said to be volatilised as a result of the heat applied to remove the contaminant from the solid matrix without the combustion of the media or contaminants (Wang *et al.*,2012). Therefore, to control air emissions, the desorbed contaminants are treated in the off-gas treatment system (ITRC, 1998).

Chang and Yen (2006) argued that usually, inorganic mercury is present in the soil in elemental form as mercury (II) compounds such as HgS, HgO, and $HgCO_3$. The mercury compounds are converted into gaseous elemental Hg when the temperature reaches 600-800 °C, which can then be recovered. Consequently, several thermal desorption experiments have reported the removal of mercury and/or decrease the mercury concentrations in the soil sample. For example, from 217 mg $kg^{-1}$ to 10 ng $g^{-1}$ after 4 hours of roasting at 700 °C (Massacci *et al.*, 2000; Huang *et al*., 2011) Hg removal efficiency ranged from 4.5% to 76%. Also, from 12.1% to 87% when the low-temperature solar furnace (LT-UPC) was operated at a temperature ranging between 28 and 280 °C, while the middle-temperature solar furnace (MT-PSA) operated with the temperatures ranged between 20 °C and 502 °C, respectively when solar energy was used to remediate mercury-contaminated soils and mine wastes from Valle del Azogue and Bayarque mines in Spain respectively (Navarro *et al.*, 2009).

While there is the success of the thermal desorption technique to remove or decrease Hg concentration, including the effective extraction and recovery of mercury, and safety. However, limitation of this method is due to the high temperatures used, this poses threat to the ecological health at the treated sites (USEPA, 2007) due to the risks from Hg being vaporised. Further, this adversely alter the soil properties with the loss of essential nutrition elements in soil such as nitrogen (N), phosphorus (P) and potassium (K) and thus render the soil unvegetated media. In addition, thermal desorption is known for the high cost of energy and the effectiveness only at rather high total soil Hg concentrations (Kucharski *et al.*, 2005).

### 2.5.5 Nanotechnology

Nanotechnology has been defined as the usage of the particle for understanding and control of matter at dimensions between approximately 1 and 100 nanometres to affect the toxicity, mobility, and bioavailability of contaminants in their natural environment. For mercury, iron sulphide (FeS) nanoparticles had been used to immobilise mercury in sediment. This was evident as reported by Xiong *et al.* (2009) that nanoparticles at a molar ratio of 26.5 (FeS-to-Hg) reduced the concentration of Hg leached into the water by 97% was thereafter reduced by 99% in a mercury-contaminated substrate.

Contrary to other technologies discussed above regarding the cost and energy, nanotechnology remediation is a low cost, low energy demand, and *in-situ* treatment application. However, before nanotechnology can be regarded as a viable remediation technique this method requires field condition testing because the potential effects of nanoparticles on the environment have not been fully investigated. Furthermore, iron nanoparticles have also been reported that may not travel far from injection point (USEPA, 2008) and the type of contaminants may limit the effectiveness of the nanoparticles. In other words, nanoparticles' remediation of the contaminant is selective.

### 2.5.6 Soil washing

This is an *ex-situ* treatment method particularly for metals to separate the contaminants from the soil through three major procedures namely physical, chemical leaching, or physiochemical procedures (Cynthia and David, 1997; Dermont *et al*., 2008). In addition, Wang *et al.* (2012) mentioned this method applicability depends on the form of metal in the waste being remediated. Therefore, metals that exist in the ionic form are reported to be applicable for chemical extraction. Particulate metals may be resolved with physical separation and/or in combination with chemical leaching (Dermont *et al*., 2008). This is because physical separation is primarily based on mineral processing technologies such as size separation, gravity concentration, froth floatation, and magnetic separation.

In the study by Sierra *et al.* (2010), physical separation has been reported to be used effectively to remediate soil contaminated with pyrite ash, which contained As, Pb, Cd, Ni, Cu, and Hg from mining and metallurgical waste in Spain. The authors reported that grain-size metals below 125 µm were successfully separated by hydro cyclone techniques, more particularly to physically separate Hg and As from soil. While grain-size coarser than 125 µm require to be milled to below 125 µm before the treatment.

The advantages of this method include; the processes allow the recycling of the extracted metals, the treated soil can be returned to the site, and the process duration is typically short to medium term in contrast to other metal extraction methods. However, the disadvantages of soil washing are not limited to the cost-effectiveness for soil with clay and silt content below 30 – 50% (USEPA, 1997b), but also difficulty with soils containing high clay and humic content. Soil washing also requires a high consumption of water for making up the washing solution, and the treatment and/or removal of metal-complexes in the washing solution must be done before the water can be safely discharged.

### 2.5.7 Electro-Remediation

The Electro-Kinetic (EK) remediation is the use of a low-intensity, direct current between a cathode ($OH^-$) and an anode ($OH^+$) introduced in the contaminated soil to enable the movement of

ions, charged small particles, and water (Mulligan *et al.* 2001; Virkutyte *et al.* 2002). For $Hg^{+2}$, the anode can be made of carbon, titanium, or steel and the cathode can be made of iron or aluminium. According to He *et al.* (2015) EK remediation generally involves four processes namely electromigration (transport of changed chemical species in the pore fluid), electroosmosis (transport of pore fluid), electrophoresis (movement of charged particles), and electrolysis (chemical reaction associated with an electric current).

Due to water electrolysis, hydrogen ions ($H^+$) are said to be generated at the anode and migrate into the bulk of soil, while $OH^-$ generated at the cathode. This then enables low pH to be developed through soil causing desorption of metallic contaminants from the soil solid phases. The accumulated metallic ions at the electrode thereafter pass-through ion exchange membranes and are removed by precipitation (Wang *et al.*, 2012).

The efficacy for the treatment of soil of low hydraulic permeability has been described as one of the most important advantages of the Elector-Kinetic technique (Reddy *et al*., 2003). However, Shen *et al.* (2009) argued that the fact that Hg has a high affinity for organic matter, explains why high organic matter content in the soil will decrease the efficiency of mercury removal from soil. Thus, EK remediation is reported to be greatly disadvantaged by soil properties such as carbonates and organic matter content and soil pH. In addition, this technique also limited by acidic conditions; the amount of time required for remediation; and the interfering effect of non-target ions on remedial progress, has been described as some of the limitations of the EK technique (Virkutyte *et al.*, 2002). Above all, the use of the EK technique for Hg-contaminated soil was reported to be difficult (Cox *et al.*, 1996) because Hg has low solubility in most natural soils while the mobility of alkylated mercury is highly limited. Also, elemental Hg presence in the soil has a high electrical conductivity of 1 x 106 s $m^{-1}$ and this is said to inhibit the electro-kinetic process by reducing the steady-state voltage gradient (Virkutyte *et al.*, 2002).

### 2.5.8 Phytoremediation

Phytoremediation can be regarded as a promising technique that uses green plants and their associated microbes for environmental clean-up by degrading or removing the contaminant and

hazardous compounds from the contaminated media (USEPA, 2008; Titah *et al.*, 2013; He *et al.*, 2015). Plant uptake and accumulation in phytoremediation for metals are argued to be dependent on the microbial community interfacing the plant roots and surrounding soil (He *et al.*, 2015).

There are five phytoremediation techniques namely rhizofiltration, phytoextraction, phytostabilisation, phytovolatilization, and phytodegradation (USEPA, 2008; He *et al.*, 2015; Mulligan *et al.*, 2001). Four of the phytoremediation methods are depicted in Figure 2.1. Phytodegradation is applicable to the breakdown of organic contaminants, while phytostabilisation involves the transformation of metal contaminants within the root zone in the soil (Mulligan *et al.*, 2001). Phytovolatilization is known for transforming contaminants into volatile forms and transpiring them into the atmosphere. Phytoextraction, on the other hand, refers to the uptake of contaminants from the contaminated media by plant roots and their translocation to and accumulation in aboveground biomass (shoots, leaves, etc) (USEPA, 2007; He *et al.*, 2015).

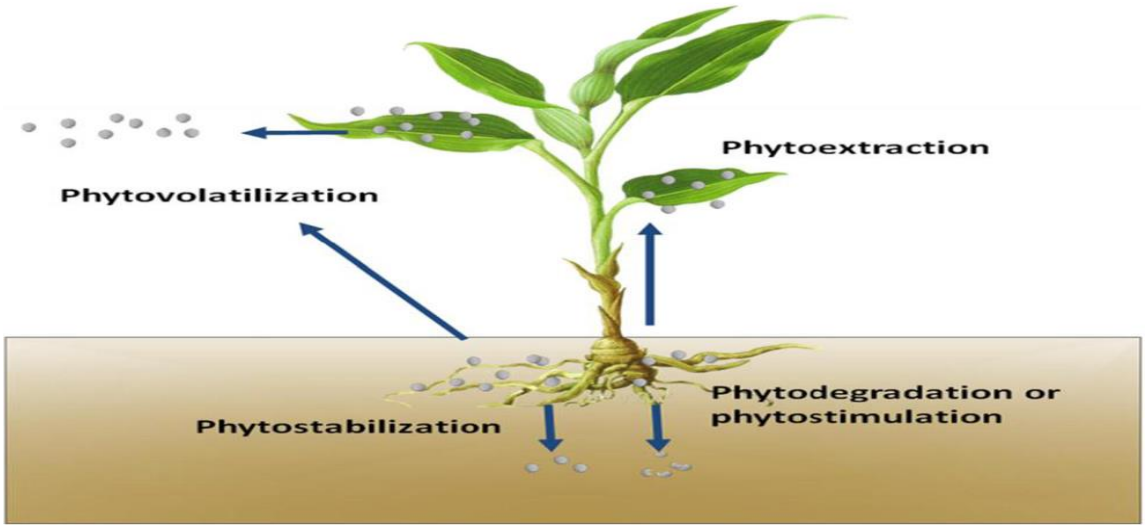



**Figure 2.1**: Illustration of the four phytoremediation processes (USEPA, 2008)

The report by Ashraf *et al.* (2013) stated that the removal of heavy metals from soil and water by plants (phytoremediation) appears to be a promising cost-effective technology. Phytoremediation can be conducted using *ex-situ* or *in-situ* methods. Suitable plants can be grown on soils or contaminated areas (non-dredged/standardised) when the *in-situ* method is used (USEPA, 2002; Ashraf *et al.*, 2013; Titah *et al.*, 2013). Phytoremediation technology, which uses green plants to

clean-up Hg contaminated media, has been reported to have many advantages over conventional methods. This includes the minimised contaminants' distribution in the soil and surrounding environment; multiple contaminants can be remediated simultaneously (ITRC, 1997); it is environmentally friendly; cost-effective; can be applied to a large area; high aesthetic pleasing value and public acceptance; and mainly required solar energy for the plants to photosynthesise (USEPA, 2000; He *et al.,* 2015).

Further, phytoremediation techniques are not only applicable with soil amendment methods to remove pollutants from the environment or decrease their toxicity (Salt *et al.,* 1998), but can also be employed with the use of rhizobacteria to provide high efficiency in remediating the contaminated media (Abou-Shanab *et al*., 2003). According to Brunetti *et al.* (2011), combining the use of rhizobacteria with compost is a suitable means for increasing the efficiency of phytoremediation. Thus, the use of compost as one of the treatments in this study to evaluate the potential of sweet sorghum in Hg-contaminated soil.

Phytoremediation is a known biological technique to remediate different media like soil, water, and sediments for organic and inorganic contaminants. Its advantages are not limited to cost effective and energetically inexpensive (Ijaz *et al*., 2016), but as well as less monitoring, environmental-friendly, long term applicability that capable to remove multiple pollutants simultaneously. Moreover, this technique has been regarded as economical, high acceptance from regulatory authorities, and less toxic by-product (Gupta *et al*., 2014).

## 2.6 Phytoextraction and hyperaccumulation potential of sweet sorghum

There is a need to distinguish between phytoextraction and hyperaccumulation. Hyperaccumulator simply refer to as the plants that are capable of concentrating heavy metals in their aboveground tissues (shoots, leaves, fruits, etc) to far exceeding levels than those present in the soil (roots) (Titah *et al.,* 2013), while phytoextraction refers to the ability of the plants to uptake the pollutants from the contaminated media by its roots and their translocation to and accumulation in aboveground biomass (Wang *et al.,* 2012). Metal hyperaccumulator plants have been reported to be useful in clean-up because they are known to take up significant amounts of metal from contaminated soils (Barker *et al*., 1994; Barman *et al.,* 2000). Utami *et al.* (2016) argued that phytoextraction efficiency of plant species is determined by two key factors, namely metal

accumulating capacity and biomass production of the plants. Thus, a plant with both high accumulation and translocation factors has been stated to have the potential to be used for phytoextraction in phytoremediation methods (Barman *et al.*, 2000; Yoon *et al.,* 2006).

Rascio and Navari-Izzo (2011) argued that the necessary features that distinguish hyperaccumulators from non-hyperaccumulator plant species are thus: heavy metals uptake; root-to-shoot translocation; and detoxification and sequestration of heavy metals. The study by Sathya *et al.* (2016) compared the heavy metal removal efficiency of various agricultural crops as hyperaccumulator plants and found sweet sorghum to have higher efficiency in heavy metal remediation compared to the other crops. Sweet sorghum is characterised by rapid growth, higher biomass, wider adaptability to harsh agroclimatic conditions including drought resistance (Du Plessis, 2008), along with its metal absorbing property, which are all propelling features to be used in phytoremediation (Zekely *et al*., 2011; Utami *et al.,* 2016; Sathya *et al.,* 2016).

Sweet sorghum (*Sorghum bicolor*) was selected for this study based on the previous studies conducted using this plant for phytoremediation of heavy metals (Luo *et al.,* 2012; Al-Chami *et al.,* 2015; Utami *et al.,* 2016; Sathya *et al.,* 2016). Sweet sorghum [ *Sorghum bicolour* (L.) Moench] is an indigenous crop to Africa, which belongs to the grass family, *Graminae*, and is recognised as a fuel for ethanol production (Sathya *et al.,* 2016). In the study by Luo *et al*. (2012). It was reported that the amendment of plant-growth-promoting (PGP) microbes (*Bacillus* sp.) promote biomass production and metals (manganese (Mn) and Cadmium (Cd)) uptake by sweet sorghum.

In the potted experiment on the effect of *Baccilus* sp. on plant growth and Mn and Cd uptake by *Sorghum bicolor*, *Phytolacca acinosa*, and *Solanum nigrum*, the plants were raised for two weeks in the nursery prior to being thinned to one plant per pot. An atomic absorption spectrometer was used for precision measurement of the metal uptakes and accumulation by the three tested plants. According to Luo *et al*. (2012), the result showed that PGP microbes not only aided the metal uptake but also significantly increased the biomass of the three tested plants for Mn and Cd uptake. The authors further stated that among the three plants, *S. bicolor* showed the highest biomass followed by *P. acinosa,* and *S. nigrum*. Hence, in the presence of 2000 mg $kg^{-1}$ of Mn

and 50 mg kg $^{-1}$ of Cd, the total Mn and Cd uptakes in the shoots of sweet sorghum were 62.5% / 40.0%, 55.2% / 31.1%, and 18.6% / 25.6%, respectively. This was in contrast to the control, unaided PGP plants. The results, as stated by Luo *et al*. (2012) showed the symbiotic relationship of *Bacillus sp.* with *S. bicolor* and the potential of *S. bicolor* as a hyperaccumulator plant species to remediate and accumulate Mn/Cd in contaminated soil. This means that sweet sorghum could be utilised for two purposes: phytoremediation of metal-polluted soil and for planting as a biofuel feedstock for ethanol production. This could ease the stress and/or problem associated with limited fertile farmland between the energy crops and food crops (Luo *et al*., 2012; Al-Chami *et al*., 2015; Sathya *et al*., 2016).

Similarly, Sathya *et al.* (2016) stated that sweet sorghum was cultivated on the contaminated lands with heavy metals to be remediated because phytoremediation method helps to preserve the natural physical and biological properties of soil. Sweet sorghum was chosen for the experiment because it is characterised by rapid growth, higher biomass potential, wider adaptability to hash agroclimatic conditions (Du Plessis, 2008). Also, sweet sorghum is known for metal-absorbing property (Luo *et al.*, 2012; Utami *et al.*, 2016; Sathya *et al.*, 2016), which made the plant to be successfully used as a phytoremediation tool. The study was correlated with other phytoremediation studies in the sense that the study was conducted to ascertain the possibilities of using sweet sorghum in the presence of Rhizobium, Pseudomonas, and Bacillus as PGP microbes to remediate the heavy metal (HM) contaminated land. The results of the study showed that sweet sorghum with PGP microbes was not able to enhance plant growth only, but also able to alleviate the stress and toxicity of HMs caused in sweet sorghum. Thus, the cultivation of sweet sorghum on HM contaminated land for phytoremediation and production of bioethanol being an of additional economical (cost-effective) advantage to reduce gasoline imports or use which is a welcoming development (Sathya *et al*., 2016).

Furthermore, a field experimental study of mercury accumulation in gold mine tailing by sweet sorghum inoculated with chromium uptake was remediated with enhanced rhizobacteria (Utami *et al.*, 2016). It was reported that the combination of sweet sorghum with the inoculated *Agrobacterium tumefaciens* was investigated for sweet sorghum growth, uptake, and accumulation of Hg from gold tailing. According to Utami *et al.* (2016), the results showed that sweet sorghum

grew well, and the plant growth was promoted by the inoculation of the rhizobacteria. Additionally, rhizobacteria increased the Hg concentrations in both stem and shoot of sweet sorghum. This is because Hg accumulation in sweet sorghum without *A. tumefaciens* was 6.2 µg per plant compared to 14.0 µg per plant in the presence of *A. tumefaciens.* Therefore, the phytoremediation efficiency of Hg in gold mine tailing was estimated to be 414 and 934 mg $ha^{-1}$ for sweet sorghum without and with *A. tumefaciens,* respectively. This confirms findings from studies by other researchers that stated that the addition of rhizobacteria enhanced the growth of sweet sorghum and alleviated the stress of Hg toxicity **(**Luo *et al*., 2012; Utami *et al.,* 2016; Sathya *et al.,* 2016**)**.

Ashraf *et al.* (2013) used bioconcentrations factors (BCFs) to determine the phytoextraction capacity of nine plant species namely *Cyperus rotundus* L., *Imperata cylindrica*, *Lycopodium cernuum, Melastoma malabathricum, Mimosa pudica* Linn, *Nelumbo nucifera, Phragmites australis* L., *Pteris vittata* L., and *Salvinia molesta* at an old tin-mining catchment. These species were selected according to previous studies conducted by the authors that assessed the phytoextraction potential for remediation of lead (Pb), copper (Cu), zinc (Zn), arsenic (As), and tin (Sn) from contaminated tin tailings. It was reported that *Cyperus rotundus L*., accumulated 658 mg $kg^{-1}$, *Imperata cylindrica* accumulated 245 mg $kg^{-1}$, *Nelumbo nucifera* accumulated 288 mg $kg^{-1}$, *Phragmites australis L.,* accumulated 345 mg $kg^{-1}$ and *Pteris vittata L.* accumulated 278 mg $kg^{-1}$ (dry weight) with bioconcentration factors (BCFs) up to 0.40, 0.32, 0.57, 0.71 and 0.65, respectively. Therefore, the phytoextraction rates of *Cyperus rotundus L*. for Sn was 86%; *Imperata cylindrica* for Zn was 42%; *Nelumbo nucifera* for As was 56%; Phragmites *australis* L. for Cu was 49%; and *Pteris vittata L.* for Pb was 31% were recorded. The study concluded that candidate plant species could successfully be used for phytoremediation of mining tin tailings in Peninsular Malaysia based on the BCFs. Considering that, this study will also make use of the BCFs as one of the parameters to determine the phytoextraction capacity of sweet sorghum and will also examine the translocation factors (TFs).

In a controlled greenhouse experiment, Marrugo-Negrete *et al*. (2015) investigated the phytoremediation potential of *Jatropha curcas* plant species in Hg-contaminated soils. The contaminated soils were of different concentration levels namely 0, 1, 5, and 10 µg Hg $g^{-1}$. The

potted experiment was carried out in 2 kg of soil. Mercury nitrate solution [Hg $(NO_3)_2$: 50 g $l^{-1}$] was used for the study. *Jatropha curcas* hyperaccumulation capacity were evaluated based on the plant growth behaviour; mercury accumulation; translocation (TF), and bioconcentration (BCF) factors. The result showed that *J. curcas* has accumulation of Hg in decreasing order: roots > leaves > stems. It was also reported that the highest cumulative absorption of Hg occurred between the second and third months of exposure, hence maximum TF detected were 0.79 and 1.04 respectively, for the different Hg concentrations. However, BCF was significantly higher in the fourth month as shown by different concentrations. In view of this, *J. curcas* could be regarded as promising plant species to remediate Hg-contaminated soil, since *J. curcas* species showed high BCFs and lower TFs (Marrugo-Negrete *et al*., 2015).

Another greenhouse study by Smolinska (2015) reported that a compost amendment of soil could help to increase the efficiency of the Hg phytoextraction process. Hence the study investigated the use of the commercial compost from the municipal green wastes to increase the efficiency of phytoextraction of mercury from mercury-contaminated soil by *Lepidium sativum* L. Sandy loam soil was used for the study, which was augmented by the green waste compost. Mercury (II) chloride ($HgCl_2$) in a concentration of 10 and 100 mg $kg^{-1}$ soil dry weight was used as the contaminant. Similarly, this study is in correlation with Smolinska (2015) that made use of green compost as one of the treatments to investigate the potential of sweet sorghum in Hg-contaminated soil, however with higher concentration of 39 mg $kg^{-1}$ all through. The experiment consisted of treatments as thus: uncontaminated natural soil (control) 0 mg $kg^{-1}$ of $HgCl_2$; 10 and 100 mg $kg^{-1}$ of $HgCl_2$; and soil and compost in soil/compost ratio 2:1, 3:1, and 4:1, were used in the study with each of the varying treatments ran in triplicate. The greenhouse system chosen was day/night temperatures of 22/19 ºC and photoperiod of 14 hours. The plant biometric parameters were determined by the length of roots and shoots, together with plant biomass, thus the data were collected on days 1, 5, 15, and 20 after phytoextraction was finished. This was followed by the bioconcentration factor (BCF) of Hg in the plant, which was calculated as a ratio of element concentration in plant shoots to a total element concentration in soil being growing medium.

The evaluation of Hg concentration in *L. sativum* L. was then conducted by weighing of plant roots and shoots separately to determine the biomass (wet weight), then later air-dried, grounded to

powdery to determine the Hg concentrations in plant sample. The results of the study indicated that *L. sativum* L was able to accumulate Hg, the control treatment accumulated over 90% of Hg in plant roots, while the lowest Hg accumulation was said to be observed in soil contaminated by 100 mg $kg^{-1}$ of $HgCl_2$. This correlated well with the investigations conducted by Perez-Sanz *et al*. (2012) that increasing concentration of Hg in soil negatively affect plant accumulation (Smolinska, 2015). Addition, it was also reported that phytotoxicity and plant stress were observed, which showed the limitation of the plant. The plants in contaminated soil showed visual symptoms of chlorosis and necrosis, and the plant biomass significantly decreased when the concentration of Hg in soil increased (Smolinska, 2015). However, it was reported that the higher the compost content, the lower the mercury pollution was observed in soil. Hence the Hg concentration in the growing medium by 100 mg $kg^{-1}$ 2:1 and 3:1 was 66.457 ± 0.182 and 74.262 ± 0.158, respectively.

The application of compost to Hg-contaminated soil is therefore said to increase the total accumulation by plant, hence the highest accumulation of Hg was observed in the treatment of soil and compost ratio 4:1. This implies that the capacity of the plant to accumulate Hg depends highly on soil treatment and correlated with plant biomass and biometric plant parameters. Thus, the application of compost as an amendment during assisted phytoextraction of Hg in soil contaminated by Hg is said to have a positive impact on the efficiency of the process. More also, soil enriched with compost has been recommended for the phytoextraction of Hg contaminated soil by *L. sativum* L. (Smolinska, 2015). A soil /compost ratio of 4:1 was chosen for this study because it gave the best result for the efficiency of phytoextraction of mercury-contaminated soil by *Lepidium sativum* L. as reported by Smolinska (2015).

A preliminary study was done to investigate the effects of organic (compost) and inorganic (NPK) fertilisers on the growth and accumulation of arsenic (As) by sweet sorghum to evaluate its phytoextraction capability. The experiment was conducted in potted, fertile garden soil, which was treated with 39 mg $kg^{-1}$ of inorganic arsenic (sodium arsenate dibasic heptahydrate; $Na_2HAsO_4.7H_20$). The study comprised of four phytoremediation treatments: As concentration of 0 mg $kg^{-1}$ as a control; As concentration of 39 mg $kg^{-1}$ only; As concentration of 39 mg $kg^{-1}$ + 0.2% of compost; and As concentration 39 mg $kg^{-1}$ + 0.2% NPK. It was found that *S. bicolor* has the capacity to accumulate arsenic in the As-spiked soils. Early physiological toxicity symptoms, senescent, and wilting was observed in the treatment with As only, while the other treatments (As

concentration of 0 mg $kg^{-1}$; As concentration of 39 mg $kg^{-1}$ + 0.2% of compost; and As concentration 39 mg $kg^{-1}$ + 0.2% NPK) showed no sign of toxicity. This preliminary finding suggests that due to the impact of NPK on the growth of the plant, it might influence the phytoremediation potential of sweet sorghum. This was because the addition of NPK fertiliser gave best result in uptake and accumulation of As by sweet sorghum (51.4%) than the other treatments.

This finding was in correlation with a previous study by Titah *et al.* (2013) in which the effect of applying a six-rhizobacteria consortium and nitrogen phosphate potassium (NPK) fertiliser in inorganic arsenic phytoremediation using *Ludwigia octovalvis* (Jacq.) Raven plants was investigated. The experiment was conducted in a controlled greenhouse with control of *L. octovalvis* plants with 0 mg $kg^{-1}$ As concentration and other three treatments namely As concentration of 39 mg $kg^{-1}$ only, the addition of six-rhizobacteria consortium at 2% (v/v) and, As concentration of 39 mg $kg^{-1}$ with the addition of NPK fertiliser at 0.02% (w/w). Titah *et al.* (2013) stated that the application of six-rhizobacteria consortium at 2% and NPK fertiliser at 0.02% were not just able to alleviate the toxicity of As in *L. octovalvis,* but as well able to increase the biomass weight of Raven plants. However, the addition of NPK fertiliser at 0.02% showed the best results and has the highest effectiveness of phytoremediation of 49.8% As compared with the other two treatments, i.e., As only and As with rhizobacteria at 2%. Based on these findings, this study investigated if sweet sorghum could be used as hyperaccumulator species for Hg-contaminated soil in the greenhouse (controlled environment), at the University of South Africa (UNISA), Florida campus. This is because, several studies have reported the soil amendment with compost as a suitable means to alleviate the toxicity stress of the contaminants and helps to increase the efficiency of phytoremediation (Ijaz *et al.*, 2016). Further, the soil amendment with NPK fertiliser has been described to increase the plant biomass and enhance the uptake and accumulation of pollutants in soils, which in turn leads to higher effectiveness of phytoremediation (Kumar *et al.*, 2017). Therefore, this study used sweet sorghum to assess the plant's potential in the presence of organic and inorganic fertiliser and compared their effective in decreasing the Hg in Hg-contaminated soil to ascertain the best treatment to be recommended for further study on field.

# CHAPTER 3

# 3. RESEARCH METHODS AND MATERIALS

## 3.1 Research design

In this study, a quantitative research design was used because it is a laboratory study in nature. The study aims to provide a causal study of a small number of cases (five replicates) under highly controlled (greenhouse) conditions. The results were achieved through laboratory analysis of plant parts and contaminated soils. The mode of observation and/or sources of data for the study was through structured observation and physical measurement of plant growth parameters (roots and shoots). The investigation then gathered numerical data from the observable phenomena (effects of the treatments on plant growth) that were used to be analysed by statistical techniques (Mouton, 2014).

## 3.2 The experimental design

This study was conducted in the greenhouse facilities of the University of South Africa's (UNISA) horticultural centre in Florida, Johannesburg, South Africa. This is in relation to previous phytoremediation studies in a controlled environment (Cheng *et al*., 2006; Titah *et al.,* 2013; Ibrahim *et al.,* 2013; Marrugo-Negrete *et al.,* 2015). The study was conducted in a greenhouse to ensure a controlled environment that provided a minimum of 14-hour photoperiods, was well aerated, and stable temperatures of between 27 to 30°C to stimulate vegetative growth (Du Plessis, 2008). In addition, the greenhouse also helped in preventing unauthorised access to the experiment and/or prevention of high-risk nature of Hg exposure to humans and to the environment. The greenhouse study was done to ensure a tightly controlled environment in which specific aspects such as concentration, photoperiod and temperature could be controlled. This ensured that the specific factors could be investigated without other environmental factors at play.

## 3.3 Materials

### 3.3.1 The soil

Standard potting soil was used for all the treatments throughout this study, which was obtained from Obaro, Pretoria. The *Culterra professional potting mix soil* that was used, provided the plants with improved moisture control, and ensured good drainage and aeration. It was a pest and disease-free potting soil augmented with bark and peat for addition nutrients to ensure vigorous and sustained plant growth. The potting soil used was air dried and passed through a 2-mm sieve to remove any stones and/or other plant residues. This was to ensure that there were no external remnants to alter the experiment. A total of 50 kg soil divided into four treatments of 12.5 kg soil each. Each treatment was further divided into five portions (replicates) of 2.5kg each as presented under phytoremediation experimental design.

### 3.3.2 Organic compost

The *Culterra compost* used for the study was also purchased from Obaro, Pretoria. The compost is made from green organic materials consisting leaves, grasses, branches, and other green wastes free from weeds and harmful soil pathogens. It adds beneficial soil microbes as well as macro and micro-nutrients to the soil (Sathya *et al*., 2016).

### 3.3.3 Inorganic fertiliser

Nitrogen, phosphorus, and potassium (NPK) fertiliser of 2:3:2 ratio was used as the inorganic fertiliser treatment for this study. It was purchased from Obaro, Pretoria. This was used to increase plant productivity and to ensure healthy plant growth (Titah *et al*., 2013).

### 3.3.4 Mercury

Inorganic mercury (II) chloride ($HgCl_2$) was used as the contaminant for this study. This is in correlation with previous phytoremediation studies (USEPA, 2007; Smolinska, 2015). $HgCl_2$ was purchased from Sigma Aldrich, South Africa of 99.5% A.C.S grade and used to contaminate the soil for this phytoremediation study. Further, the solubility of (HgCl2) in aqueous solutions which facilitates its movement in soil and the plant tissues informed the use of this inorganic mercury.

The Hg stock solution was prepared to spike the soils prior to the seedlings being transplanted into the pot. The stock solution was made from neat crystal $HgCl_2$ in order to have even distribution of the contaminant in the potted soils. The powdery 33.85 mg of $HgCl_2$, i.e.,13.54 mg Hg per kg soil x 2.5 kg soil per pot = 33.85 mg of $HgCl_2$, was diluted in 300 ml of deionised water under the fume hood. The solution was then heated at 277 °C on a hot plate with the aid of a magnetic stirrer for about 10 – 15 minutes, after which about 700 ml of deionised water was added to bring the solution to 1 litre. The 1 litre of $HgCl_2$ stock solution was then used to spike each pot containing 2.5 kg of potting soil, which resulted to 13.54 mg Hg concentration per kg of soil.

This concentration was lower than the 39 mg $kg^{-1}$ approved in ethical clearance (Appendix A) by the ethics committee of the College. The use of this lower concentration (13.54 mg $kg^{-1}$) was based on the concentrations used in the previous experiments carried out, both in the controlled environment and/or on the field, in Witwatersrand, South Africa as reported by Malehase *et al.* (2016) concentration to be between 0.890-6.755 µg $g^{-1}$. Also, in countries such as Thailand, Pataranawat *et al.* (2007) reported 10.5 mg $kg^{-1}$, as well as Malaysia, India, and China with lower concentrations (Feng *et al.*, 2006; Kokyo *et al.*, 2015). Hence, the study used lower concentration to the proposed concentration. Despite the lower concentrations previously used in phytoremediation studies, these concentrations were all reported to exceed the environmental unsafe level of mercury contamination in soils.

### 3.3.5 Nitric acid

The Nitric Acid ($HNO_3$) of 70% volume used was purchased from Sigma Aldrich, South Africa. The acid was used for the process of Hg extraction from both plants and soils for the phytoremediation study. Nitric acid was also used for acid-washing during the microwave digestion for the study.

### 3.3.6 Sweet sorghum (*Sorghum bicolor*)

The seeds of sweet sorghum used for the study were obtained from Obaro, Potgietersrus. Sweet sorghum is a type of grass indigenous to Africa that is grown for its grains used for flour, made into beer, syrup, and is also used as animal feed. The plant was used in previous phytoremediation studies (Zhuang and Shao, 2009; Al Chami *et al.,* 2015; Sathya *et al*., 2016).

## 3.4 Analysis of soil properties

The properties of the soil and geochemical characteristics such as soil pH, electrical conductivity (E.C.), moisture content, total carbon, total nitrogen, total organic carbon and other mineral elements, namely Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K), Sodium (Na), Iron (Fe), Zinc (Zn), Manganese (Mn), Aluminium (Al), Boron (B) and Copper (Cu) in the soil were analysed. The experiments and instruments used are as thus: all these analyses were carried out at the laboratory of Agricultural Research Council (ARC), Institute for Soil, Climate and Water. Arcadia, Pretoria. The sample was prepared for the analysis based on the methods adopted by the Institute for Soil Climate and Water (ARC-ISCW). The results of the analysis of soil nutrients and properties are presented in Table 4.1 under discussions and result in chapter 4.

The sample was divided into two portions and each portion was weighed into and spread out in a pre-weighed flat container so that the sample depth was approximately 5 to 10 mm, and left standing at room temperature until they appeared dry (about four days) and mass fairly constant. The air-dried portions were weighed and the percentage moisture loss on air-drying was calculated from the

difference in mass before and after air-drying, divided by the sample mass before air-drying (x 100%). The portions were combined, milled, and mixed to obtain homogeneity. The formula is adapted from method 967.03 B Method II (AOAC, 1980) as expressed in the below equation:

$$\frac{\text{Mass before air-drying} - \text{mass after air-drying}}{\text{Sample mass before air-drying}} \times 100 \qquad \text{---------- equation 1}$$

### 3.4.1 Soil pH

The pH of the soils was determined by weighing about 10 g of the soil samples into a beaker and 50 ml deionised water was added to it. Sample was stirred (30 seconds) and allowed to stand for 30 minutes, stirring every 5 minutes. The pH was measured, using a pH electrode and pH meter calibrated against buffers at pH 4 and pH 7 and checked against a pH 10 buffer.

This method gives a 1:5 (mass/volume) ratio, which is the most dilute of the commonly used ratios for soil pH (Handreck and Black, 1994) and is more concentrated than the 1:10 ratio used for inorganic fertilisers (Hignett, 1991) or the 3:50 ratio recommended for Peat (AOAC, 1980). Using the more dilute 1:10 ratio usually gives a pH value slightly (0.2 average) higher than the standard 1:5 ratio, while a more dilute ratio was also found to give slightly higher pH values for soil (Handreck and Black, 1994).

### 3.4.2 Electrical conductivity (E.C.)

The air-dried sample was weighed into a bottle. Then 50 ml deionised water was added, and samples were shaken (wrist-action mechanical shaker) for 30 minutes, thereafter centrifuged. The conductivity was measured with a suitable conductivity electrode and meter.

The E.C. is much more sensitive than the pH to sample: water ratio, so it is essential to specify the sample: water ratio. If the sample: water ratio is doubled (twice as concentrated) then the E.C. will typically increase between 50% and 90% (maximum 100%), depending on the solubility of the major soluble salts. The following ratio 1:10 (mass/volume – air dried) was used in this laboratory

as the standard ratio requiring 5 g of air-dried samples. It is suitable for all compost samples, including those that are water absorbent (Thomas and Ward, 1993).

### 3.4.3 Moisture, total solids, ash, and organic carbon determination

The samples were determined in triplicate. For each determination, approximately 5 g of sample was weighed into a porcelain bowl and dried in an oven at 70 °C for more than 3 hours. The sample was cooled, covered with Aluminium (Al) foil to prevent moisture absorption from the atmosphere, and then weighed. The percentage of moisture in the sample was calculated as the mass loss during drying divided by the original sample mass (x100%).

Recall from Equation I:

Let **y** = % moisture loss on air-drying

a = % moisture in air-dried sample (A.D. moisture)

Therefore, Total moisture% = y + a (1-y/100).

The total solids content is calculated as 100% - total moisture% (AOAC, 1980).

For organic carbon measurements, the same sample was placed in pre-heated furnace set at 550°C and left for another hour. The organic material was thus burnt up, leaving the ash behind. After cooling (covered with Al foil), the porcelain bowls with the ash were weighed and the ash content was calculated as a percentage of the original sample (moist sample). The organic matter is calculated as the mass lost during ashing. Therefore, on a moist sample basis:
Organic matter = total solids – ash. On air-dried basis: Organic matter = 100 - A.D. moisture – ash.

### 3.4.4 Method for C (carbon) and N (nitrogen) determination

The air-dried sample was used directly (in a powdery form) for C and N determinations on a Thermo Scientific Flash 2000 Elemental Analyzer, using between 3 and 12 mg sample weighed

into a tin foil container for each determination. (Jimenez and Ladha, 1993). The method is a dry oxidation method generally known as the Dumas method.

The sample and tin container are ignited at high temperature (950°C) in oxygen (on a chrome oxide catalyst) to produce carbon dioxide, nitrogen gas, and oxides of nitrogen (plus other oxides, etc). The gases produced pass through silvered cobalt oxide (to remove oxides of Sulphur and Halogens) and a column of Copper (650 to 680°C), which reduces the oxides of nitrogen-to-nitrogen gas (and removes excess free $O_2$). After removal of water vapour by a trap of anhydrous magnesium perchlorate, the $N_2$ gas and $CO_2$ are finally separated by gas chromatography (GC), using a helium carrier gas and detected by a thermal conductivity detector (TCD).

The "Eager Xperience" software is used to control the instrument, integrate, calibrate (linear or quadratic) and compute the N and C concentrations (from the peak areas). The values for C and N are on an air-dried basis and may be converted back to the sample as received (moist), or to a dry basis if necessary. It should be noted that, values converted to a dry basis may be higher than those obtained from the direct determination on oven-dried samples, due to probable losses of N in the form of ammonia and possibly C in the form of $CO_2$ during oven drying.

The instrument is calibrated against a pure organic compound of known composition (certified standard). The compound chosen for our calibration standard is phenylalanine (an amino acid), which contains 8.48% N and 65.4% C.

### 3.4.5 Method for perchloric + nitric acid sample digestion

The sample was digested in triplicate, using 1 g of the air-dried sample with 7 ml $HNO_3$ (concentrated nitric acid) and 3 ml $HClO_4$ (perchloric acid) for each digest. The temperatures increased to 180°C, and a few drops of $H_2O_2$ (hydrogen peroxide) were added. After cooling and addition of 10 ml 1:1 HCl, each digest solution was brought to volume in a 100 ml volume flask. This method was adapted from Zasoski and Burau (1977).

### 3.4.6 ICP-OES determination of eleven mineral elements

The instrument is set up and operated according to the recommended procedures in the instrument manual. Since all elements are determined simultaneously, it is not possible to optimise each individual element, but only for the group of elements. The instrument is calibrated against a series of standard solutions, containing all the elements of interest in the proportions found in typical compost samples. This method was developed and optimised by ARC-ISCW based on the recommended procedures in the instrument manual and user guide.

An aliquot of each digest solution was analysed using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) to determine the concentrations of Ca, Mg, P, K, Na, Fe, Zn, Mn, Al, B, and Cu. The instrument used is an Agilent 725 (700 Series) simultaneous instrument, where all the elements (and all wavelengths) are determined simultaneously. Each element was measured at one or two appropriate emission wavelengths, chosen for high sensitivity and lack of spectral interferences. The wavelengths used were, namely Mg:383.829 and 279.553 nm; Ca:422.673 and 317.933 nm; K:766.491 nm (and 769.897 nm); P:213.618 nm; Na:589.592 nm; Fe:259.94 and 238.204 nm; Mn:257.61 nm; Zn:213.857 nm; Cu:324.754 & 327.395 nm; B 249.678 (and 249.772) nm and; Al 396.152 (and 308.215) nm. Background correction on one side or both sides of the peak was used where necessary. The results of properties of the potting soil used are presented in results and discussion.

## 3.5 Phytoremediation experimental design

### 3.5.1 The Summary of the experimental design, sample and data collection

The summary of the experimental design sampled and data collection for this study is depicted in Table 3.1. All samples were collected in triplicate randomly in 5 pots (n = 15) per treatment.

At every 20 days exposure after transplanting, one plant per pot was sampled randomly for roots and stems length; wet-dry weight; analysis of Hg bioavailable concentrations in the Hg-spiked soil; and 13.54 mg $kg^{-1}$ concentration of Hg up-taken and accumulated in the whole sampled plants through translocation and bioconcentration factors.

**Table 3.1**: Summary of the experiment design, sampled and data collection

| Treatments | Five Replicates |
|---|---|
| 0 mg $kg^{-1}$ Hg concentration (Control) | Three plants per pot<br>n=15 |
| 13.54 mg $kg^{-1}$ Hg concentration only | Three plants per pot |
| 13.54 mg $kg^{-1}$ Hg concentration + 0.25% Compost | Three plants per pot |
| 13.54 mg $kg^{-1}$ Hg concentration + 0.2% NPK | Three plants per pot |

### 3.5.2 Propagation of plant species

The emergence test was conducted with the sweet sorghum (*Sorghum bicolor*) seeds that were used for the study, prior to being planted for the phytoremediation process. This was to ensure the seeds would germinate if planted. Ten seeds were tested for the emergence test in a petri-dish, laid and covered with damp cotton wool that was moistened with distilled water. The seeds were placed directly on the cotton wool in the petri-dish in triplicate and then put in an oven at room temperature. The result of the emergence test was considered successful after five days if there was a 90% average germination achieved and was calculated by using this equation:

$$\frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100\%$$

The seeds were later grown in the greenhouse with a minimum of 18-hour photoperiod and 19°C, in the nursery tray of 27 cm x 27 cm x 10 cm. The seedlings were watered every 2-3 days) with equal quantities to ensure a constant moisture content. The healthy individual seedlings of the same average height were selected from the nursery and transplanted after three weeks of planting into

pots of 15 X 35 cm. Each pot contained three plants at 10 cm depth and three replicates for each control and contaminated soil with various phytoremediation treatments were used (Table 3.1).

### 3.5.3 Addition of organic compost

The soil to compost ratio was 4:1 weight percent of the green compost (comprise of leaves, grasses, branches, and other green waste) was added as organic compost to the Hg-spiked soil because of their high content of organic matter (Smolinska, 2015). The total mass of compost added to the soil was 625 g (0.25%) per pot of 2.5 kg of soil, hence the ratio of 4:1 of soil to compost used. This was mixed thoroughly to compare its effect on mercury uptake and accumulation in the study. Compost was used in this study based on the previous studies (Cao and Ma, 2004; Smolinska, 2015) and for the enhancement of plant growth-promoting (PGP) microbes in phytoremediation (Sathya *et al*., 2016). The compost was mixed with the soil and was divided into five replicates in the pots. The soil was spiked with Hg stock solution, and then transplanted *S. bicolor* seedlings unto the spiked soil.

### 3.5.4 Addition of inorganic fertiliser

Nitrogen, phosphorus, and potassium (NPK) fertiliser of ratio (2:3:2) was used in this study. This was based on previous studies (Titah *et al.*, 2013; Utami *et al.*, 2016). The total mass of soil used for this treatment was 12.5 kg, which was mixed thoroughly with 25 g of NPK fertiliser or 0.2% of the total weight of the soil. These soil mixtures were then divided into five replicates in the pot prior to being spiked with the Hg stock solution and then transplanted sweet sorghum seedlings.

### 3.5.5 Treatment with Hg-spiked soil in the pot

The soil was spiked with inorganic mercury (II) chloride ($HgCl_2$) stock solution. The stock solution was prepared by dissolving 33.85 mg of $HgCl_2$ in 1 litre of water to give a final concentration of 33.85 ppm. The 1 litre of water was then added to 2.5 kg of soil in the pot to achieve 13.54 mg Hg per kg of soil. Control sweet sorghum plants were used in addition to the three phytoremediation treatments in *S. bicolor* plants, namely Hg concentration of 13.54 mg $kg^{-1}$ only; the addition of

organic compost (0.25%) with Hg concentration of 13.54 mg $kg^{-1}$; and the addition of NPK fertiliser of 0.2% (w/w) with Hg concentration of 13.54 mg $kg^{-1}$ (Table 3.1). The transplanted plants were watered with 300 ml of clean water every 3-5 days and the moisture content of the soils was kept constant in the controlled greenhouse environment throughout the exposure period. At every 20 days interval of exposure after transplanting, one plant per treatment was sampled for root and stem lengths to determine the height of the plants in different treatment; wet-dry weight; analysis of Hg concentrations in the Hg-spiked soil; and concentration of Hg uptake and accumulation in the whole sampled plants as illustrated in Table 3.1.

## 3.6 Sampling and analysis of plants and soils

Plant sampling was done after the designated exposure interval of 20, 40, and 60 days after transplanting. Three plants were sampled by digging up the whole plant using a plastic scoop and pulled up slowly to prevent any plant parts from being trapped and/or left in the pot. The sampled plants were then washed to remove soil remnants in the roots, weighed (wet-weight), measured, and recorded for all four treatments to compare the plant heights and physiological effects on the growth of the plants.

Similarly, sampling of the soil was done on day 20; 40; and 60 after transplanting into Hg-spiked soil. Triplicate samples of about 10 g of soil were taken around the plant roots of each soil treatment 13.54 mg $kg^{-1}$ Hg concentration only; 13.54 mg $kg^{-1}$ of Hg concentration + 0.25% compost; and 13.54 mg $kg^{-1}$ of Hg concentration + 0.2% NPK using grab sampling techniques (Feng *et al*., 2006; Pataranawat *et al*., 2007; Titah *et al*., 2013; Malehase *et al.,* 2016) and air dried at room temperature. The plants and soil samples were then placed in clearly labelled and sealed plastic bags and transferred to the laboratory (Botany and Environmental Sciences, UNISA Florida Campus) where they were stored at -20 degrees Celsius until further analysis. The soils were stored under this freezing temperature to maintain the integrity of the samples collected. Also, intended to retard biological action, retard hydrolysis of chemical compounds and complexes, and reduce volatility of constituents.

## 3.7 Laboratory analyses

### 3.7.1 Plant extraction

Plant parts (roots, stems, and leaves) were dried in the oven at 60 °C for 72 hours (Brunetti *et al.*, 2011**)** prior to the extraction procedure. The plant parts (roots and shoots) were separately weighed for comparisons of wet-dry weights of the plants for the four treatments, dried and then crushed into fine powder.

The extraction of Hg from sweet sorghum was performed according to EPA method 3051A (USEPA, 2007). Approximately 0.1 – 0.5 g of the plant material (dry weight) was microwave digested using 10 ml of 69% $HNO_3$ solution to extract the acid-soluble portion of the metal. Each sample was run in triplicate and the microwave power program was set to 100% with the temperature held at constant 180 °C for 20 minutes. After cooling of the vessels, 5% $HNO_3$ was used for acid washing. The samples were firstly filtered through acid-washed Whatman 0.45 µm filter paper to remove any particulates and was later filtered through the RESTEK syringe filter of 0.45 µm, to further remove any finely particulate before ICP-OES analysis. The filtered sample was brought to 50 ml in a volumetric flask, and then transferred into a 50 ml plastic tube, deionised water was added to bring the volume of the sample to 50 ml. The Hg concentrations in plant parts i.e., roots and shoots were determined using a Shimadzu ICP-OES (SHIMADZU, ICPE-9820). The data obtained (Appendix B) was used to determine the translocation factor of Hg from soil to the roots and the aerial parts of the plants.

### 3.7.2 Soil extraction

Soil extraction was carried out to determine bioavailable Hg by using the methods of Quevauviller (1998) as described in Titah *et al.* (2013). Large particles and lumps were crushed, and all the visible plant residues were removed. Each soil sample was finely ground with a pestle and mortar in the laboratory before the extraction process commenced.

A 5 g soil sample from each of the four treatments was taken for extraction using composite sampling method (Xing and Yeneman, 1998). Each soil sample was placed in a conical flask and

then 10 ml 69% $HNO_3$ was added to each flask in a fume hood. Samples were then agitated using an orbital shaker at 30 rpm for 1 hour at room temperature (Titah *et al.*, 2013). Thereafter, each sample was centrifuged at 3 000 rpm for 10 minutes. The samples were filtered, through Whatman filter paper, Number 40, 9 cm diameter (Whatman, UK) with the pore size 20 – 25 µm and then with a syringe filter with a pore size 0.45 µm. The solution was collected in plastic tubes of 50 mL and stored at 4 °C before being analysed by using an ICP-OES for the concentration of bioavailable Hg.

### 3.7.3 Translocation and bioconcentration factors

The TF and BCF factors of Hg were estimated, based on the determined Hg concentration levels in plant parts and soil. The TF for Hg in a sweet sorghum plant was calculated as the ratio of Hg concentration in the shoots to the roots (Zu *et al*., 2005), while the BCF was expressed as the ratio of Hg concentration in the plant to that in soil (Barman *et al*., 2000; Yoon *et al*., 2006; Smolinska, 2015). Plants with both BCFs and TFs greater than one (TF and BCF > 1) has been described and reported to have the potential to be used in phytoextraction in phytoremediation (Barman *et al*., 2000; Zu *et al*., 2005; Yoon *et al*., 2006; Smolinska, 2015).

## 3.8 Statistical data analysis and interpretation

The results were presented as the mean ± standard deviation of triplicate determinations. The two-way analysis of variance (ANOVA) test and mean comparison by using Duncan Multiple Range Test for pairwise comparisons were performed among the treatments when needed using SPSS (version 26) software. The significance of 95% ($p \leq 0.05$) confidence level was selected, to ensure reliability and validity of the data analysed, in order to evaluate significant changes and/or comparisons in the wet-dry weight of *S. bicolor* biomass, length of roots and shoots, bioavailable concentrations of Hg in the spiked soil and concentration of Hg taken-up in whole *S. bicolor* plants.

It should be noted that the ANOVA tables comprised of degree of freedom (df), which indicated the sample size minus the number of parameters needed to calculate for each of the statistical analysis. Also, F-values indicate if the means analysed between populations are significantly

different. Where the F-value is significant, the Duncan Multiple range tests is conducted. High F-value indicates that the group means spread out more than the variability of the data within groups (Leedy and Ormrod, 2015). Thus, it becomes more likely that the observed differences between group mean reflect differences at the population level. However, low F-value indicates that group means cluster together more tightly than the within-group variability (Frost, 2020). R-squared and/or adjusted R-squared is the percentage of the dependent variable variation that a linear model explains. It measures the strength of the relationship between the model and the dependent variable. The data for this study yielded a low R-squared value but the variables are statistically significant and this help to draw important conclusions about the relationships between the variables.

The Duncan multiple range comparison has almost similar standard error because the means for groups are homogeneous subsets as analysed by the SPSS software (IBM SPSS Statistics Version 26). Based on observed means, each Duncan multiple range comparison therefore displayed the mean square (error) by using Harmonic Mean Sample size at the bottom of each comparison table alongside with their significant difference indicated with an asterisk (*) at alpha = 0.05.

## 3.9 Ethical consideration, quality assurance, and quality control

The research ethical clearance was applied for which was considered and approved (ERC Reference #: 2018/CAES/015) by the College of Agriculture and Environmental Sciences (CAES) Health Research committee. The CAES Health Research committee is registered with the National Health Research Ethics Council (NHREC Registration #: REC-170616-051). All the ethical standards for this study were considered to ensure that not only the environment is at risk of being damaged intentionally or accidentally but also the researcher was not at risk. This was achieved by the researcher avoiding every possible contact with the chemical (Hg), spiked and/or contaminated soil, plant species (*S. bicolor*) by wearing protective clothing (goggle, exhaust for breathing protection, gloves) during work. Also, eating or drinking was prohibited during work and hands were washed after completing any practical work.

Extra care was taken to avoid spilling and contamination to the environment during and after the experiment. The plant species and the soils were placed in sealed plastic, clearly labelled as “toxic waste” placed in the laboratory waste for proper disposal at the end of the experiment.

The plant species that were used for this study, sweet sorghum, is not among the South African National Biodiversity Institute (SANBI) protected plants but has a status of “least concern” (Fish and Victor, 2005).

The quality assurance (QA) of the study was ensured by maintaining quality in all aspects of the research program ranging from the viability and emergence test of the seeds, seeds nursery raising and transplanting to the Hg-spiked soil, sampling, data collection, and data analysis, to the data evaluation (Zweiebel, 2005). Hence, the data was analysed with a 95% confidence level ($p \leq 0.05$). Furthermore, standardised soil was used for all the treatments, with accurate measurement of the soil and Hg prior to being mixed with measured 500 ml of Hg stock solution. This was to ensure that the data collected meet defined standards of quality with a standard level of confidence.

Further, quality control (QC) of the study, which is referring to the routine technical activities and established checks and balances for the purpose to control error (USEPA, 1996) was considered. Thus, the samples were analysed at the accredited and standard laboratories namely, Agricultural Research Council, Institute for Soil, Climate and Water (ARC - ISCW), Arcadia Pretoria, and UNISA Botany and Environmental laboratory, Florida campus. This helped to ensure the data met defined standards of quality with the level of confidence in QA.

# CHAPTER 4

# 4. RESULTS AND DISCUSSION

## 4.1 Physicochemical properties of potting soil

The analysis of physicochemical properties of experimental soils used as illustrated in Table 4.1, which include the eleven mineral elements in the soils was determined by ICP-OES. The result showed that amongst the concentrations of Ca, Mg, P, K, Na, Fe, Zn, Mn, Al, B, and Cu analysed, the concentration of phosphorus in the soil was lower when compared to other macronutrients for plant growth and nutrition in the soil. Hence, the use of NPK fertiliser of phosphate high proportion of 2:3:2 to supplement the potting soil used.

**Table 4.1:** Analysis of chemical properties of potting soil

| Element | Conc. A.D. | Conc. Wet | Conc. Dry | Units |
|---|---|---|---|---|
| pH * | 5.86 | | | |
| Elec. Conductivity* | 63.5 | | | mS $m^{-1}$ |
| Moisture | 5.06 | 51.0 | | % |
| Tot. Solids | 94.94 | 49.0 | | % |
| Moisture Lost | 48.4 | N.A. | | % |
| Ash | 229 | 118 | 241 | g $kg^{-1}$ |
| Organic Matter | 721 | 372 | 759 | g $kg^{-1}$ |
| Total C | 393 | 203 | 414 | g $kg^{-1}$ |
| Total N | 5.64 | 2.9 | 5.9 | g $kg^{-1}$ |
| C/N Ratio | 69.7 | | | |
| Organic Carbon Ratio | 1.83 | | | |
| P | 0.775 | 0.400 | 0.816 | g $kg^{-1}$ |
| K | 2.46 | 1.27 | 2.59 | g $kg^{-1}$ |
| Na | 403 | 208 | 425 | mg $kg^{-1}$ |
| Ca | 8.98 | 4.64 | 9.46 | g $kg^{-1}$ |
| Mg | 3.37 | 1.74 | 3.55 | g $kg^{-1}$ |
| Fe | 9.47 | 4.89 | 9.98 | g $kg^{-1}$ |
| Cu | 13.2 | 6.8 | 13.9 | mg $kg^{-1}$ |
| Mn | 752 | 388 | 792 | mg $kg^{-1}$ |
| Zn | 114 | 59 | 120 | mg $kg^{-1}$ |
| B | 9.1 | 4.7 | 9.6 | mg $kg^{-1}$ |
| Al | 6.15 | 3.17 | 6.47 | g $kg^{-1}$ |

A.D. is air dried (room temperature).
C, N and other elements were determined on air dried samples.
Values in the conc. wet column have been calculated back to the samples as received (wet).
Moisture Lost is the moisture lost on air-drying.
* The sample (A.D.) to water ratio used is 1 g:5 ml for pH and 1 g:10 ml for electrical conductivity.

This was in correlation with previous studies (Brunetti *et al.*, 2011; Truex *et al.*, 2010) in which soil pH, electrical conductivity (EC), total organic carbon (TOC), and soil nutrients were analysed. The results suggest that the potting soil used was rich in organic matter due to the mixture of peat by the *Culterra professional potting mix soil* for additional nutrients.

## 4.2 Physiological growth of sweet sorghum

Symptoms of mercury toxicity such as stunted growth and inhibition of photosynthesis, senescence in leaves, wilting, and necrosis were observed in the potted *Sorghum bicolor* plants, despite all the environmental conditions (stable temperature and 14-hour photoperiods) being met. This is consistent with the symptoms of Hg toxicity observed in *Lepidium sativum* plant reported by Smolinska (2015). The plants with Hg treatment only were more susceptible to the Hg toxicity compared to the other treatments with organic and inorganic fertilisers i.e., the effect of Hg contamination in soil was more obvious in physiological growth of the plants in treatment with Hg only until the last day of exposure. Nagajyoti *et al.* (2010) argued that Hg ions in plants during photosynthesis, may substitute the other essential nutrients ions present in soil and terminate the photosynthetic process by inhibiting the electron transport chain where Photosystem-II (PS-II) is the main target. Thus, the inhibition of the enzymes involved in the biosynthesis of chlorophyll led to the decrease in chlorophyll content in sweet sorghum in Hg only treatment (Marrugo-Negrete *et al.*, 2016). Furthermore, exposure to Hg has also been reported to alter the photosynthetic apparatus, especially at the donor site of PS-II, the oxygen-evolving protein, and the β-subunit of adenosine triphosphate (ATP) synthase in the chloroplast (Nicolardi *et al.*, 2012).

Based on the observation of the physical growth of *S. bicolor*, no toxic effects of Hg were observed until the end of exposure in the treatment with the addition of 0.2% NPK fertiliser. The addition of NPK fertiliser probably increased the biomass wet-weight of *S. bicolor* at the end of the exposure duration as shown in Figure 4.1. The addition of NPK fertiliser was more than seven-fold increase (17.72 g) compared to the biomass in phytoremediation with Hg only (1.7 g). Further, there was no obvious toxic effect of Hg on *S. bicolor* planted on the at the 13.54 mg $kg^{-1}$ Hg concentration dosed 4:1 compost soil compared to the Hg concentration only partial inhibition of growth was observed in *S. bicolor* planted on the treatment with the addition of compost compared to the phytoremediation treatment of Hg + NPK fertiliser.

All the sweet sorghum plants grew well until the end of exposure in the treatment with the addition of compost. The average dry-weight biomass of *S. bicolor* at the termination of exposure on soil with the addition of compost was the second highest (2.5 g) as shown in Figure 4.2. This may probably be due to the addition of compost that inhabitant the PGP microbes that stimulate the plants growth. Sathya *et al.* (2016) argued that PGP microbes stimulated the growth of sweet sorghum, and enhanced plant growth in their study of cultivation of sweet sorghum on heavy metal contaminated soils by phytoremediation approach for the production of bioethanol. This was also in support of several phytoremediation studies conducted showing similar results. Rhizobacteria as PGP was mentioned to be responsible for the plant growth facilitation (Khan *et al*., 2009). In addition, Titah *et al.* (2013) argued that the wet-weight biomass of *L. octovalvis* plants in the presence of PGP in arsenic phytoremediation study was higher than that of the *L. octovalvis* plants with the As only.

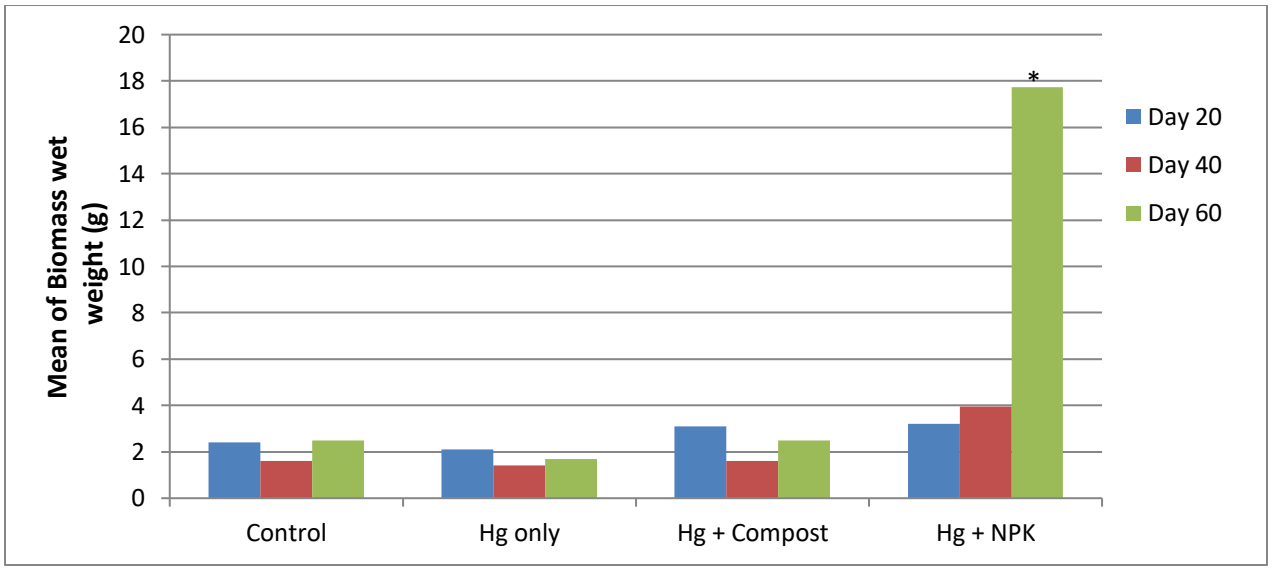



**Figure 4.1**: Wet-weight biomass of *S. bicolor*.

Statistically significant difference of the mean weight indicated by a*.

Mercury (Hg) concentrations in all treatments were 13.54 mg $kg^{-1}$ soil.

The cumulative mean weight was the highest in the treatment with the addition of 0.2% NPK fertiliser compared to the others three treatments as depicted in Figure 4.1. The mean difference is significant at the 0.05 level, as illustrated in Table 4.2(a).

**Table 4.2(a)**: ANOVA for wet-weight biomass of *S. bicolor*

Dependent Variable: Wet-weight

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Model | 147.759[a] | 4 | 36.940 | 9.468 | .004 |
| Treatments | 147.759 | 4 | 36.940 | 9.468 | .004 |
| Error | 31.213 | 8 | 3.902 | | |
| Total | 178.972 | 12 | | | |

a. R Squared = .826 (Adjusted R Squared = .738)

In addition, the results as shown in Table 4.2(b) of Duncan pairwise multiple comparisons indicate that the wet-weight biomass of *S. bicolor* in the treatment with NPK fertiliser shows a significant difference ($p < 0.05$) against the control and other two treatments as depicted in Table 4.2(b).

**Table 4.2(b):** Duncan Pairwise Comparisons for wet-weight biomass of *S. bicolor*

Dependent Variable: Wet-weight

| | (I) Treatments | (J) Treatments | Mean Difference (I-J) | Sig. | 95% Confidence Interval Lower Bound | 95% Confidence Interval Upper Bound |
|---|---|---|---|---|---|---|
| LSD | Control | Hg only | .1667 | .920 | -3.5524 | 3.8858 |
| | | Hg + Comp | -.6333 | .705 | -4.3524 | 3.0858 |
| | | Hg + NPK | -3.5533 | .059 | -7.2724 | .1658 |
| | Hg only | Control | -.1667 | .920 | -3.8858 | 3.5524 |
| | | Hg + Comp | -.8000 | .633 | -4.5191 | 2.9191 |
| | | Hg + NPK | -3.7200* | .048 | -7.4391 | -.0009 |
| | Hg + Comp | Control | .6333 | .705 | -3.0858 | 4.3524 |
| | | Hg only | .8000 | .633 | -2.9191 | 4.5191 |
| | | Hg + NPK | -2.9200* | .050 | -6.6391 | .7991 |
| | Hg + NPK | Control | 3.5533 | .059 | -.1658 | 7.2724 |
| | | Hg only | 3.7200* | .048 | .0009 | 7.4391 |
| | | Hg + Comp | 2.9200* | .050 | -.7991 | 6.6391 |

Based on observed means.

The error term is Mean Square (Error) = 3.902.

*. The mean difference is significant at the 0.05 level.

This was correlated with the ANOVA results on the dry-weight biomass of *S. bicolor* as shown in Table 4.3(a). This indicated that there was a significant difference ($p = 0.007$) in the treatment with the addition of 0.2% NPK fertiliser against the other three treatments.

**Table 4.3(a):** ANOVA for dry-weight biomass of *S. bicolor*

Dependent Variable: Dry-weight

| Source | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Model | 114.287[a] | 4 | 28.572 | 7.987 | .007 |
| Treatments | 114.287 | 4 | 28.572 | 7.987 | .007 |
| Error | 28.618 | 8 | 3.577 | | |
| Total | 142.905 | 12 | | | |

a. R Squared = .800 (Adjusted R Squared = .700)

There was no significant difference in the dry weight of samples taken on day 20 (Figure 4.2). This suggested that indeed phytoremediation of Hg by sweet sorghum with no treatment, succumbed to the inhibition of photosynthesis and growth, and the toxicity of mercury in sweet sorghum as exposure period increase, while the treatment with the addition of 0.2% NPK fertiliser alleviated the inhibition and toxicity of mercury as the exposure period increases.

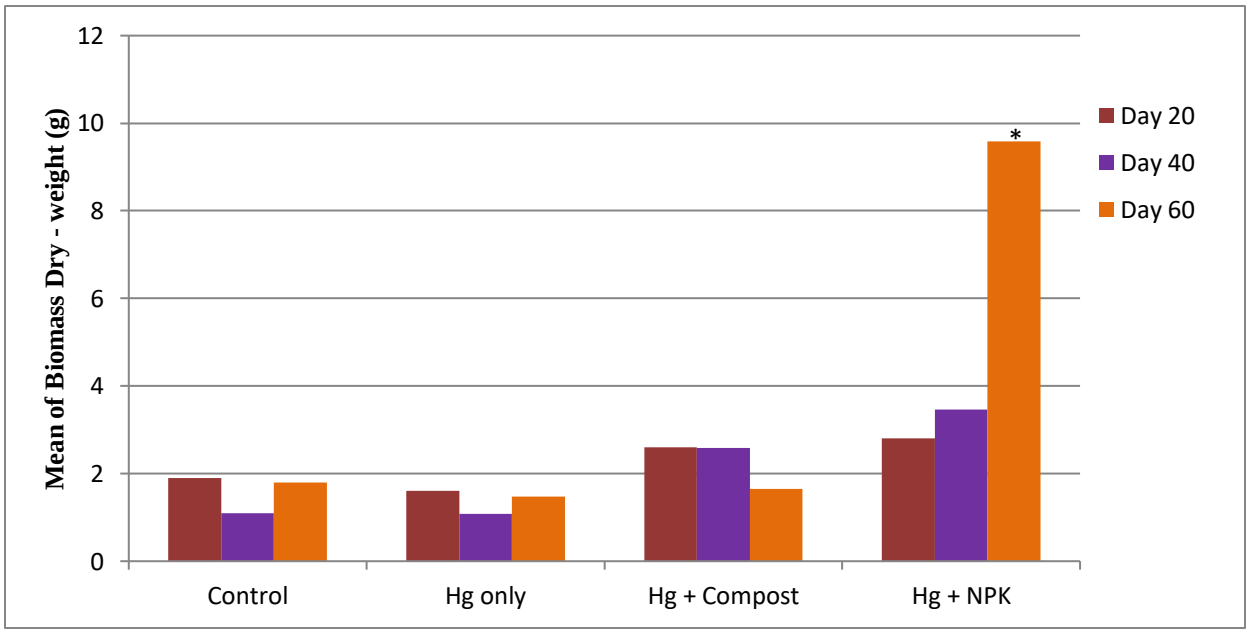



**Figure 4.2**: Dry-weight biomass of *S. bicolor*.

Statistically significant difference indicated with an * ($p = 0.007$).

Mercury (Hg) concentrations in all treatments were 13.54 mg $kg^{-1}$ soil.

In order to ascertain if there is a significant difference between the treatments, Duncan multiple range test was conducted and the results as shown in Table 4.3(b) indicates that a significant difference exists between the treatment with the addition of 0.2% NPK against control ($p = 0.042$); treatment with Hg only ($p = 0.033$), while there was no significant difference between the treatment with the addition of 0.2% NPK fertiliser against the treatment with the addition of compost and control ($p > 0.05$).

Furthermore, all the plants were healthy, green leaves and grew well until the average height of the plant shoot reached 55.33 cm (Figure 4.3), while the average root length of *S. bicolor* in this treatment was also the highest (42.83 cm) at day 60 as shown in Figure 4.4. This result confirms that the addition of NPK fertiliser could not only reduce the toxicity effects of Hg but also aid in alleviating the inhibition of plant growth (Gulz, 2002).

**Table 4.3(b):** Duncan Pairwise Comparisons for dry-weight biomass of *S. bicolor*

Dependent Variable: Dry-weight Biomass of *S. bicolor*

| | | | Mean Difference | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | (I) Treat | (J) Treat | (I-J) | Sig. | Lower Bound | Upper Bound |
| LSD | Control | Hg only | .2300 | .885 | -3.3312 | 3.7912 |
| | | Hg + Comp | -.6600 | .680 | -4.2212 | 2.9012 |
| | | Hg + NPK | -3.7433* | .042 | -7.3045 | -.1822 |
| | Hg only | Control | -.2300 | .885 | -3.7912 | 3.3312 |
| | | Hg + Comp | -.8900 | .580 | -4.4512 | 2.6712 |
| | | Hg + NPK | -3.9733* | .033 | -7.5345 | -.4122 |
| | Hg + Comp | Control | .6600 | .680 | -2.9012 | 4.2212 |
| | | Hg only | .8900 | .580 | -2.6712 | 4.4512 |
| | | Hg + NPK | -3.0833 | .081 | -6.6445 | .4778 |
| | Hg + NPK | Control | 3.7433* | .042 | .1822 | 7.3045 |
| | | Hg only | 3.9733* | .033 | .4122 | 7.5345 |
| | | Hg + Comp | 3.0833 | .081 | -.4778 | 6.6445 |

Based on observed means.

The error term is Mean Square (Error) = 3.577.

*. The mean difference is significant at the 0.05 level.

This is in correlation with the preliminary and previous studies in which the addition of NPK in a phytoremediation study was reported to reduce the toxic effects of Arsenic in *L. octovalvis* and *S. bicolor* in greenhouse experiments conducted (Titah *et al.*, 2013).

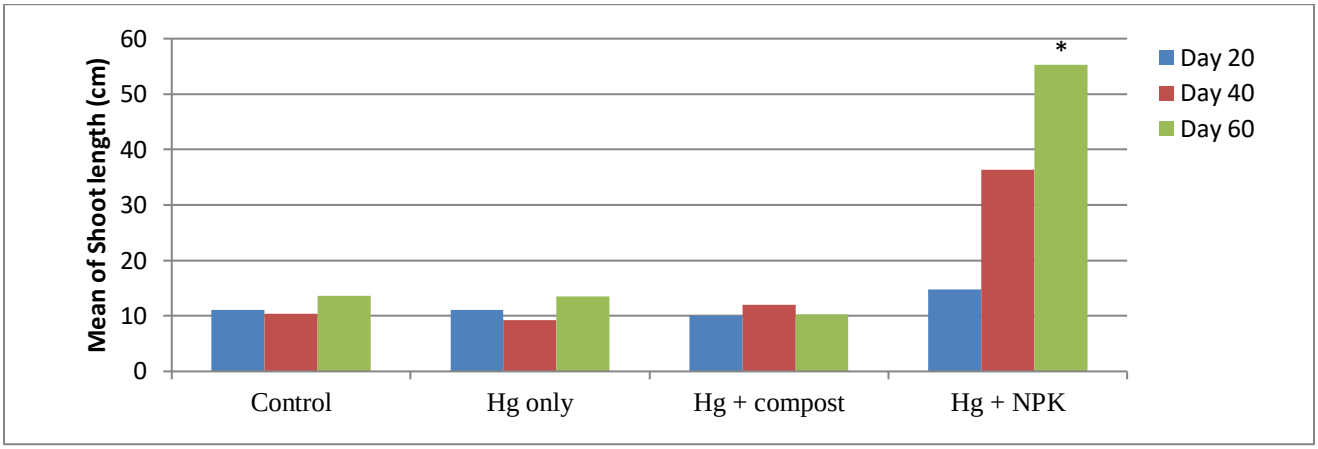



**Figure 4.3**: Shoot length of *S. bicolor*.

Mercury (Hg) concentrations in all treatments were 13.54 mg $kg^{-1}$ soil and were statistically different with a *p*-value of 0.002.

The ANOVA results as depicted on Table 4.4(a) on the shoot length of sweet sorghum shows that the mean difference is significant ($p < 0.05$).

**Table 4.4(a):** ANOVA for shoot length of *S. bicolor*

Dependent Variable: Shoot-length

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Model | 4915.967[a] | 4 | 1228.992 | 11.666 | .002 |
| Treatments | 4915.967 | 4 | 1228.992 | 11.666 | .002 |
| Error | 842.797 | 8 | 105.350 | | |
| Total | 5758.764 | 12 | | | |

a. R Squared = .854 (Adjusted R Squared = .780)

In addition, Duncan pairwise comparisons between the treatments as shown in Table 4.4(b) suggests that the treatment with the addition of 0.2% NPK fertiliser is significant against the control and the other two treatments.

**Table 4.4(b):** Duncan Pairwise Comparisons for Shoot length of *S. bicolor*

Dependent Variable: Shoot-length

| | (I) Treatments | (J) Treatments | Mean Difference (I-J) | Sig. | 95% Confidence Interval Lower Bound | 95% Confidence Interval Upper Bound |
|---|---|---|---|---|---|---|
| LSD | Control | Hg only | .4333 | .960 | -18.8922 | 19.7588 |
| | | Hg + Comp | .9100 | .916 | -18.4155 | 20.2355 |
| | | Hg + NPK | -23.8000* | .022 | -43.1255 | -4.4745 |
| | Hg only | Hg only | -.4333 | .960 | -19.7588 | 18.8922 |
| | | Hg + Comp | .4767 | .956 | -18.8488 | 19.8022 |
| | | Hg + NPK | -24.2333* | .020 | -43.5588 | -4.9078 |
| | Hg + Comp | Control | -.9100 | .916 | -20.2355 | 18.4155 |
| | | Hg only | -.4767 | .956 | -19.8022 | 18.8488 |
| | | Hg + NPK | -24.7100* | .018 | -44.0355 | -5.3845 |
| | Hg + NPK | Control | 23.8000* | .022 | 4.4745 | 43.1255 |
| | | Hg only | 24.2333* | .020 | 4.9078 | 43.5588 |
| | | Hg + Comp | 24.7100* | .018 | 5.3845 | 44.0355 |

Based on observed means.
The error term is Mean Square (Error) = 105.350.

*. The mean difference is significant at the 0.05 level.

Moreover, the ANOVA results in Table 4.5(a) also show that the mean difference of root length was highly significant ($p < 0.05$).

**Table 4.5(a):** ANOVA for root length of *S. bicolor*

Dependent Variable: Root-length

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Model | 7766.848[a] | 4 | 1941.712 | 74.459 | .000 |
| Treatments | 7766.848 | 4 | 1941.712 | 74.459 | .000 |
| Error | 208.621 | 8 | 26.078 | | |
| Total | 7975.468 | 12 | | | |

a. R Squared = .974 (Adjusted R Squared = .961)

Figure 4.4 illustrated the average mean of root length until the last day of exposure in which Hg concentrations in all treatments were 13.54 mg $kg^{-1}$ soil.

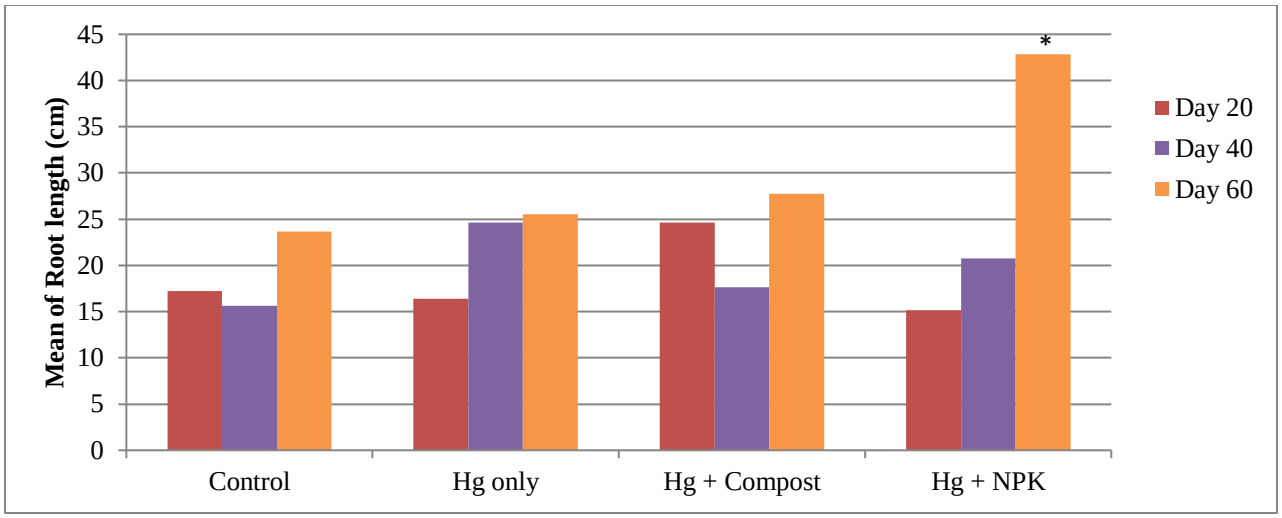



**Figure 4.4:** Root length of *S. bicolor*.

Mercury (Hg) concentrations in all treatments were 13.54 mg $kg^{-1}$ soil and the mean difference was statistically highly significant with a *p*-value of 0.000.

Moreover, there was a significant difference ($p < 0.05$) in the root length of sweet sorghum with the addition of NPK fertiliser against all other treatments when analysed and compared in Duncan multiple range test as shown in Table 4.5(b).

**Table 4.5(b):** Duncan Pairwise Comparisons for root length of *S. bicolor*

Dependent Variable: Root-length

| | (I) Treatments | (J) Treatments | Mean Difference (I-J) | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| LSD | Control | Hg only | -4.5367 | .308 | -14.1516 | 5.0783 |
| | | Hg + Comp | -2.2800 | .599 | -11.8950 | 7.3350 |
| | | Hg + NPK | -16.3667* | .004 | -25.9816 | -6.7517 |
| | Hg only | Control | 4.5367 | .308 | -5.0783 | 14.1516 |
| | | Hg + Comp | 2.2567 | .603 | -7.3583 | 11.8716 |
| | | Hg + NPK | -11.8300* | .022 | -21.4450 | -2.2150 |
| | Hg + Comp | Control | 2.2800 | .599 | -7.3350 | 11.8950 |
| | | Hg only | -2.2567 | .603 | -11.8716 | 7.3583 |
| | | Hg + NPK | -14.0867* | .010 | -23.7016 | -4.4717 |
| | Hg + NPK | Control | 16.3667* | .004 | 6.7517 | 25.9816 |
| | | Hg only | 11.8300* | .022 | 2.2150 | 21.4450 |
| | | Hg + Comp | 14.0867* | .010 | 4.4717 | 23.7016 |

Based on observed means.
The error term is Mean Square (Error) = 26.078.
*. The mean difference is significant at the 0.05 level.

Based on these results, it can be inferred that the type of fertilisers (NPK and green compost) used could have effects on the phytoremediation capability of sweet sorghum in Hg-contaminated soil. This is because, phytoremediation treatment with the addition of 0.2% NPK fertiliser gave the best result on the physical and physiological growth of *S. bicolor*, given the fact that all the plants with the treatments were statistically significantly all other treatments.

## 4.3 Bioavailable concentration of mercury in soil

The bioavailable concentrations of mercury in the spiked soil are shown in Figure 4.5. The average mean concentrations of bioavailable Hg in the treatment with the addition of 0.2% NPK fertiliser was the lowest (0.77 mg $kg^{-1}$) when compared with the other two treatments, i.e., Hg + Comp (0.84 mg $kg^{-1}$) and Hg only (0.90 mg $kg^{-1}$) at the end of the duration (60 days). ANOVA results (Table 4.6) suggests that the difference in the mean concentrations of bioavailable Hg in the NPK treatment was statistically significant ($p<0.05$) when compared to the other two treatments. These findings are supported by several previous studies in which mercury concentrations in the contaminated media were significantly reduced due to the presence of organics such as phosphates (Rieser *et al.*, 2001; Randall and Chattopadhyay, 2010). Soils rich in organics have shown high capacity for sorption of metals. As a result, Hg might sorb strongly to the soil, which will inadvertently reduce the quantity of the bioavailable Hg that can be taken up by the plant.

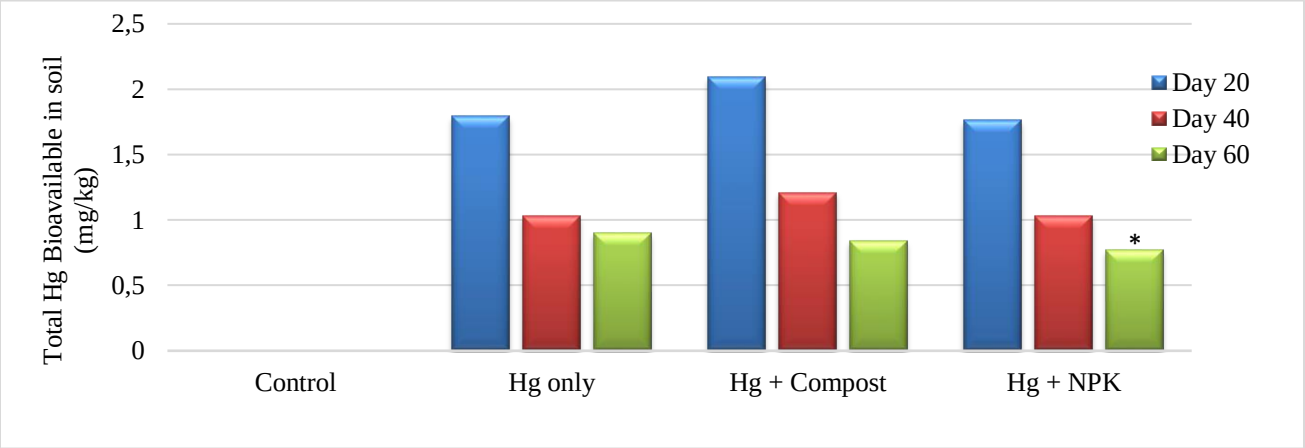



**Figure 4.5**: Total Hg bioavailable in soil.

Mercury (Hg) concentrations in all treatments were 13.54 mg $kg^{-1}$ soil and statistical difference ($p < 0.03$) are indicated with an * with a *p*-value of 0.003.

It should be noted that the difference between the bioavailable concentrations of Hg amongst the treatments during the exposure period (day 20 and day 40) were not significant. The Hg bioavailable concentrations in the treatment with the addition of compost was less (0.84 mg $kg^{-1}$) than the initial concentrations the plants were exposed to. Although the Hg bioavailable concentration of the compost treatment was lower than the Hg only soil after 60 days, it was not statistically significant. According to Lehmann and Kleber (2015) the type of soil used and the amount of quality organic matter in the soil might affect the effectiveness of the phytoremediation process. Phosphates are most often successfully used in stabilisation or solidification of mercury contaminated media to reduce the mobility of mercury (He *et al.*, 2015). Khan *et al.* (2009) also noted that the presence of PGPR microbes can reduce the concentration of bioavailable heavy metals.

**Table 4.6:** ANOVA for bioavailable Hg concentration in Hg-contaminated soil

Dependent Variable: Hg Bioavailable

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Model | 14.551[a] | 3 | 4.850 | 16.045 | .003 |
| Treatments | 14.551 | 3 | 4.850 | 16.045 | .003 |
| Error | 1.814 | 6 | .302 | | |
| Total | 16.364 | 9 | | | |

a. R Squared = .889 (Adjusted R Squared = .834)

Despite the short exposure period, the lowest bioavailable concentrations of Hg recorded in the treatment with the addition of 0.2% NPK fertiliser on day 60 of exposure of sweet sorghum to mercury in mercury-contaminated soil was less than the minimum standards for the remediation of contaminated land. According to the National Environmental Management: Waste Act, 2008 (Act No. 59 of 2008) of the Department of Environmental Affairs (DEA, 2012), the national soil screening norms and standards for the remediation of contaminated land and soil quality for mercury is 0.93 mg $kg^{-1}$. The value is protective of both human health and eco-toxicological risk for multi-exposure pathways, inclusive of contaminant migration to the water resource. In this study, sweet sorghum in the presence of compost and NPK fertiliser was able to reduce the level

of Hg contamination to levels lower than the national norms with NPK having a better effect. in Hg-contaminated soil.

## 4.4 Translocation and bioconcentration factors

In order to ascertain if sweet sorghum can be used as hyperaccumulator plant species for the remediation of Hg in Hg contaminated soil in a controlled environment, the translocation and bioconcentration factors were determined. The potential of sweet sorghum to translocate Hg from the roots to the aerial parts (shoots) of the plants called the translocation factor (TF) was calculated as the ratio of Total Hg in the shoot to the Total Hg in the root (Table 4.7(a)). The relative TFs for different exposure times after transplanting sweet sorghum into the Hg-contaminated soils were not in uniformity of increasing and/or decreasing order from day 20, day 40, and day 60. As reported by Marrugo-Negrete *et al.* (2015), the translocation of Hg from roots to the aerial parts of the plant occurs when the TF is higher than 1. This is regarded as a value that characterised accumulator and/or hyperaccumulator plant species (Baker and Brooks, 1989; Tu and Ma, 2003; Ali *et al.*, 2013).

**Table 4.7(a):** Translocation factor (TF)

| Phytoremediation treatment | TF of *Sorghum bicolor* in Hg-contaminated soil | | |
|---|---|---|---|
| | Day 20 | Day 40 | Day 60 |
| 13.54 mg $kg^{-1}$ Hg only | 1.07 | 1.69 | 1.02 |
| 13.54 mg $kg^{-1}$ Hg + 0.25% compost | 1.00 | 1.05 | 1.10 |
| 13.54 mg $kg^{-1}$ Hg + 0.2% NPK | 1.00 | 1.48 | 1.55 |

All the TFs of the three phytoremediation treatments were higher than 1 on the last day of exposure. The ANOVA analysis of the translocation factors was also found to be significant different ($p < 0.05$) as shown in Table 4.7(b).

**Table 4.7(b):** ANOVA for translocation factor of *S. bicolor* in Hg-contaminated soil

Dependent Variable: TF

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Model | 13.567[a] | 3 | 4.522 | 71.520 | .000 |
| Treatments | 13.567 | 3 | 4.522 | 71.520 | .000 |
| Error | .379 | 6 | .063 | | |
| Total | 13.947 | 9 | | | |

a. R Squared = .973 (Adjusted R Squared = .959)

The results indicated that the phytoremediation treatment with the addition of NPK fertiliser had the highest (1.55) TF on day 60. The TFs for this study on the last day of exposure were in decreasing order of NPK > Compost > Hg only. It was noted that the TF values for all the treatments on day 40 were higher than day 20 and then dropped in day 60, given in bell shape uptake progression. The results suggest that the uptake of nutrients and contaminants from soil by plants is age related, i.e., there is that age where uptake by plant is high and there is that age when it is slow. As noted in this study, juvenile plants have high uptake characteristics as they need the nutrients for growth, while older plants uptake less. This may be regarded as one of the reasons phytoremediation as a technique is time bound for effective removal of contaminants. This suggests that most of the Hg were translocated in *S. bicolor* at the beginning of the exposure and then less was available and therefore translocated at the end of the exposure. This contrasted with the study by Natasha *et al*. (2020) in which it was reported that most of the Hg taken up by plants is retained in the root and less is translocated towards the shoot. This depends on the plant and soil physicochemical parameters as well as climatic condition (weather). One possible reason to explain the higher Hg translocation that occurred in NPK treatment was probably the high phosphate in NPK fertiliser of 2:3:2 used that alleviated the stress of Hg toxicity and enhanced the physiological growth of *S. bicolor* to the extent of having higher wet-dry weight biomass than the other three treatments. This result showed that the treatment with the addition of 0.2% NPK fertiliser did alleviate the Hg stress and toxicity for sweet sorghum in Hg-contaminated soil. The results further affirm the effectiveness of *S. bicolor* to be viable for mercury uptake and accumulation in Hg-contaminated soils.

The Bioconcentration Factor (BCF) was expressed as the ratio of Hg concentration in the plant to that in soil. In this study, the BCF values were only higher than 1 after 60 days of exposure as shown in Table 4.8(a), and this is consistent with other researchers that reported that plants with both TF and BCF > 1 can be regarded to have the potential to be used in phytoextraction for phytoremediation method (Barman *et al*., 2000; Yoon *et al*., 2006; Smolinska, 2015). The BCF values for all the three treatments, in this study, were lower than 1 as at Day 20 and 40. The phytoremediation treatment with the addition of green compost had the highest BCF (0.95) on day 40. Thus, the decreasing order as of Day 40 of exposure to Hg-contaminated soil was Hg + Comp > Hg only > Hg + NPK. However, the reverse is the case on the last day of exposure as the phytoremediation treatment with the addition of NPK has the highest BCF (1.32) and the decreasing order changed to Hg + NPK > Hg only > Hg + Comp (Table 4.8 (a)). Therefore, *S. bicolor* showed maximum removal capacity for Hg at day 60. This implies that when using this plant as a phytoextractor, good removal efficiency will be at day 60 and possibly beyond, as significant proportion of the soil Hg is removed with BCF and TF > 1.

**Table 4.8(a):** Bioconcentration factor (BCF)

| Phytoremediation treatment | BCF of *Sorghum bicolor* in Hg-contaminated soil | | |
|---|---|---|---|
| | Day 20 | Day 40 | Day 60 |
| 13.54 mg $kg^{-1}$ Hg only | 0.59 | 0.76 | 1.18 |
| 13.54 mg $kg^{-1}$ Hg + 0.25% compost | 0.49 | 0.95 | 1.16 |
| 13.54 mg $kg^{-1}$ Hg + 0.2% NPK | 0.50 | 0.66 | 1.32 |

This was in correlation with the TF values as treatment with the addition of NPK fertiliser has the highest BCF against the other two treatments. This means that sweet sorghum in NPK treatment could be classified as Hg resistant because it did not exhibit any sign of toxicity and has the capacity to uptake Hg from the Hg-contaminated soil. Both the TF and BCF values were > 1 indicating good metal transport towards the aerial parts of the plant on day 60. Therefore, both TF and BCF values for *S. bicolor* indicate that a high probability that Hg was significantly transferred from soil to the plant (Marrugo-Negrete *et al.*,2015).

The BCF was also found to be statistically different in ANOVA analysis as illustrated in Table 4.8(b). In view of this, sweet sorghum, as the results suggested, can be used and/or could be

considered as hyperaccumulator plant species for Hg-contaminated soil since the TFs and BCFs > 1 due to its phytoextraction capability although it is better effective in the presence of NPK fertiliser (Barman *et al*., 2000; Zu *et al.*, 2005; Yoon *et al*., 2006; Marrugo-Negrete *et al.*,2015).

**Table 4.8(b):** ANOVA for BCF of *S. bicolor* in Hg-contaminated soil

Dependent Variable: BCF

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Model | 6.437[a] | 3 | 2.146 | 16.149 | .003 |
| Treatments | 6.437 | 3 | 2.146 | 16.149 | .003 |
| Error | .797 | 6 | .133 | | |
| Total | 7.234 | 9 | | | |

a. R Squared = .890 (Adjusted R Squared = .835)

## 4.5 Mercury uptake and accumulation

As shown in Table 3.1, *S. bicolor* plants were sampled after 20, 40, and 60 days of transplanting into the Hg-contaminated soils. In order to give an accurate assessment of the phytoremediation performance of sweet sorghum, the uptake concentration by the plant was converted to Total Hg uptake to indicate the effectiveness of either of the three phytoremediation treatments (Hg only; Hg + compost; Hg + NPK) by using the equation given in Titah *et al*. (2013) as shown below to yield total percentage uptake as thus:

$$\text{Total Hg uptake (\%)} = \frac{\text{CHg in plant x DW plant x N plant}}{\text{C bioavailable Hg in soil x W soil}} \times 100 \quad \text{---------- equation 2}$$

where,

CHg in plant = concentration of Hg uptake per plant (mg kg $^{-1}$)
DW plant = dry weight of plant (Kg)
N plant = number of plants
C bioavailable Hg = concentration of Hg bioavailable in soil (mg kg $^{-1}$)
W soil = total weight of soil (Kg)

These results are shown in Figure 4.6 and based on these results, in relation to the Hg spike in the soil, the effectiveness of the phytoremediation on day 60 of exposure were 23.56%, 26.43%, and

84.31% in treatment with Hg only; treatment with the addition of compost and the treatment with the addition of NPK fertiliser, respectively. This was in correlation with the residual concentrations in soil when compared with bioavailable concentrations as depicted in Figure 4.5. The calculations for total percentage mercury uptake are shown in Appendix C.

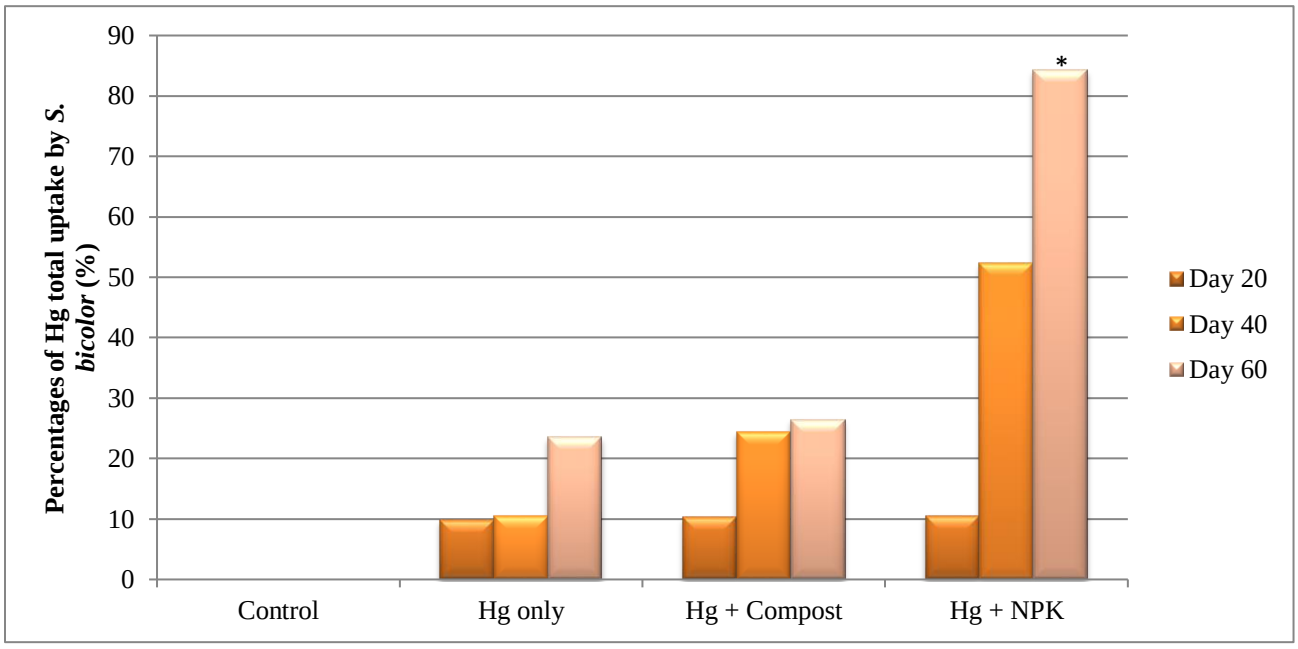



**Figure 4.6:** Percentages of Hg total uptake

The ANOVA analysis showed that there was a significant difference at the 0.05 level as depicted in Table 4.9.

**Table 4.9:** ANOVA for Total % Hg uptake of *S. bicolor* in Hg-contaminated soil

Dependent Variable: Total % Hg Uptake

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Model | 9109.565[a] | 3 | 3036.522 | 6.049 | .030 |
| Treatments | 9109.565 | 3 | 3036.522 | 6.049 | .030 |
| Error | 3011.845 | 6 | 501.974 | | |
| Total | 12121.411 | 9 | | | |

a. R Squared = .752 (Adjusted R Squared = .627)

The results suggest that the accumulation of mercury in Hg-contaminated soil through the phytoremediation method is viable and proved *S. bicolor* to be potentially suitable for phytoremediation of Hg-contaminated soil. The best Hg phytoremediation application was the treatment with the addition of 0.2% NPK fertiliser since the total Hg uptake by *S. bicolor* had the highest effectiveness of the phytoremediation at 60 days after transplanting into Hg-contaminated soils. This may due to the presence of phosphates which is the highest portion of NPK fertiliser used and the high concentration of potassium in the potting soil as outlined in Table 4.1. This result is in agreement with previous study by Gonzaga *et al.* (2006) in which the presence of phosphate in contaminated soils was reported to have a role in the phytoextraction process of phytoremediation. Furthermore, the uptake of heavy metals in the presence of phosphate by plants has also been argued to be generally competitive. The results of total percentage of Hg uptake in this study was in support of the previous studies that the presence of phosphates, which was the highest proportion of the NPK fertiliser of 2:3:2, likewise the possibly high concentration of potassium in the potting soil may have enhanced Hg mobility. This was evident not only significantly promoted height and biomass, but also in biosorption of Hg by plants and reduction of the Hg toxicity effects on the plants compared to the other phytoremediation treatments (Tu and Ma, 2003; Titah *et al.*, 2013). Therefore, the enhanced mobility of Hg in Hg-contaminated soil and subsequent increased plant uptake (Figure 4.6) in the treatment with the addition of NPK fertiliser may be due to the replacement of phosphate by Hg at the soil binding sites as it was reported by Gupta *et al*. (2014).

The Duncan multiple comparisons result of Hg concentrations in *S. bicolor* showed that Hg concentration was significantly different ($p < 0.05$) in the phytoremediation treatment with the addition of 0.2% NPK fertiliser compared to the other two treatments. The results further indicate that the addition of NPK fertiliser as well as green compost could facilitate the remediation of Hg-contaminated soils using sweet sorghum. This is because the phytoremediation treatment with the addition of 0.25% compost gave the second-highest total percentage uptake and accumulation of Hg. The findings of this study corroborate phytoremediation study reported by Natasha *et al*. (2020) where it was suggested that organic matter maybe regarded as one of the factors that affect Hg speciation and bioavailability. Sweet sorghum reduced the level of mercury to the lowest in the contaminated soil by the end of exposure duration in the phytoremediation treatment with the

addition of 0.2% NPK fertiliser (Figure 4.5). This also has the highest percentage uptake and accumulation of Hg as shown in Figure 4.6. This in turn means that sweet sorghum was able to reduce the level of Hg in all the treatments as depicted in Figure 4.5, even in the treatment without any compost or fertiliser. Although the addition of both compost and fertiliser improved the Hg phytoextraction capability of sweet sorghum in Hg-contaminated soil, but the treatment with the addition of NPK proved to be most effective.

Several studies have reported that Hg contamination exerts toxic effects in plants even at low applied concentrations which resulted in many defects such as inhibition of oxidation of lipid membrane (Zhou *et al*., 2008), DNA and protein damage (Malar *et al*., 2015), growth retardation (Ahammad *et al.*, 2018) and inhibition of photosynthesis (Assad *et al*., 2016), to mention few. Despite the negative effects of Hg-induced oxidative stress, Natasha *et al.* (2020) argued that plants have developed some defensive mechanisms include enzymatic antioxidants and some non-enzymatic antioxidants which were reported by several researchers to play a crucial role in the detoxification of Hg inside the plant (Meng *et al*., 2011; Zhang *et al*., 2017; Shao *et al.*, 2018).

# CHAPTER 5

# 5. CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

The investigated phytoremediation potential of sweet sorghum in mercury-contaminated soil for this study concludes as follow:

### 5.1.1 Sweet sorghum as hyperaccumulator plant species

It has been reported in several studies that plant with both bioconcentration factor and translocation factor greater than 1 has a value that is characterised for heavy metal hyperaccumulator plant species. Based on the results recorded in this study, sweet sorghum was able to accumulate mercury in the aboveground tissues that were far exceeding levels present in the soil. Thus, the results indicated that both TFs and BCFs > 1 in this study due to the phytoextraction capability of sweet sorghum. This suggests that *S. bicolor* can be used and/or regarded as hyperaccumulator plant species in Hg-contaminated soil. It should however, be noted that the treatment with the addition of 0.2% NPK fertiliser had the highest value of 1.55 and 1.32 for TF and BCF, respectively, and gave the best result at termination of exposure.

### 5.1.2 Reduction of Hg concentration in the Hg-contaminated soil

The level of spiked soil with 13.54 mg $kg^{-1}$ $HgCl_2$ to examine the phytoextraction potential of sweet sorghum in the phytoremediation technique was reduced to a greater extent. As shown in Figure 4.5, the Hg bioavailable concentrations in soil at the last day of exposure period indicated that the lowest concentration was recorded in the treatment with the addition of 0.2% NPK fertiliser was 0.77 $kg^{-1}$ compared with the other two treatments higher than that: 0.84 $kg^{-1}$ (treatment with the addition of compost) and 0.90 $kg^{-1}$ (treatment with Hg only). These concentrations were lower than the South African norms and Quality Standard of Hg concentration for the remediation of contaminated land and soil quality for Hg permissible (0.93 $kg^{-1}$) concentration. Thus, the level of Hg concentration was reduced in this order NPK < Compost < Hg only.

### 5.1.3 Effects of fertiliser used on *S. bicolor* phytoextraction capability

The results of the comparison between the effect of the treatments suggest that the application of NPK fertiliser at 0.2% was the most effective to alleviate the inhibition of photosynthesis and toxic effects of Hg in *S. bicolor* as it was more conspicuous than the other treatments. The addition of NPK fertiliser also increased both wet-dry biomass weights of the plant, physiologically. NPK fertiliser addition at 0.2% during mercury phytoremediation treatment increased the effectiveness of the phytoremediation by alleviating the stress of Hg toxicity and showed the highest effectiveness of phytoextraction capability of *S. bicolor* to take-up and accumulated up to 84.31% compared with the other two phytoremediation treatments, i.e., Hg + compost (26.43%) and Hg only (23.56%). In view of this, Hg phytoremediation with the addition of 0.2% NPK fertiliser at 2:3:2 proportion gave the best results.

The significance of this study is that, host communities around gold tailing may employ this affordable method to remediate the polluted media in their environment since sweet sorghum is indigenous to Africa and multiple contaminants can be remediated simultaneously (ITRC, 1997). More especially where there is large deposit of ASGM tailing in Africa like Niger, Nasarawa and Plateau states in Nigeria (Oramah *et al.*, 2015) and Witwatersrand basin, South Africa, as reported by Malehase *et al*. (2016). This has been reported to be of benefit for such communities as sweet sorghum known as energy crop can be planted on the gold tailing also for biofuel production to serve as another source of income.

## 5.2 Recommendations

The limitation and recommendations for this study are as follows:

- There is a need for more studies to understand the extent of Hg toxicity in sweet sorghum that resulted in inhibition of photosynthesis and stunted growth of the plant in order to ascertain that Hg toxicity in plant grown on Hg-contaminated soils lead to defect in plants' physiological growth. This could be done by examining the anatomical structure of the plant's transporting system through the scanning electron microscopy (SEM) analysis of xylem and phloem tissues.

- The study was also unable to ascertain if Hg would be transported and accumulated by the plant's seeds at the end of the exposure period. Therefore, more studies also need to be done for longer period to study if the accumulated Hg could be transported into the seeds so as to prevent the contaminated seeds from sweet sorghum to enter the food chain. More importantly if sweet sorghum is directly planted gold mine tailing of artisanal gold mining (ASGM) communities to use this method to remediate the soil contaminated by ASGM waste deposited on the soil.
- The success of this study in a controlled environment means that future research should be conducted to test this technique on the field and compare the results with the controlled environment experiment.

# Appendix A: Ethical clearance

UNISA university of south africa

**CAES HEALTH RESEARCH ETHICS COMMITTEE**

Date: 28/03/2019

NHREC Registration # : REC-170616-051
REC Reference # : 2018/CAES/015
Name : Mr IO Dauda
Student #: 55094864

Dear Mr Dauda

**Decision: Ethics Approval Renewal after First Review from 01/03/2019 to 28/02/2020**

**Researcher(s):** Mr IO Dauda
55094864@mylife.unisa.ac.za

**Supervisor (s):** Mrs T Fouche
fouchtc@unisa.ac.za; 011-670-9711

**Working title of research:**

Phytoremediation potential of sweet sorghum in mercury-contaminated soil

**Qualification:** MSc Environmental Science

Thank you for the submission of your progress report to the CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is renewed for a one-year period. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

**Due date for progress report: 28 February 2020**

*The **low risk application** was **reviewed** by the CAES Health Research Ethics Committee on 01 March 2018 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

## **Appendix B**: **ICP – OES generated data for Hg concentrations in soil and plant parts**

| Sample No | Sample Cla | Sample Na | G# | | Date/Time of Analy | Hg Quant Average mg/ |
|---|---|---|---|---|---|---|
| 9 | SOIL | D20NPK1 | 1 | | 2019/11/18 12:40 | 1.92 L |
| 10 | SOIL | D20NPK2 | 1 | | 2019/11/18 12:50 | 1.82 L |
| 11 | SOIL | D20NPK3 | 1 | | 2019/11/18 13:00 | 1.55 L |
| 12 | SOIL | D20HG1 | 1 | | 2019/11/18 13:09 | 1.93 L |
| 13 | SOIL | D20HG2 | 1 | | 2019/11/18 13:19 | 1.75 L |
| 14 | SOIL | D20HG3 | 1 | | 2019/11/18 13:29 | 1.70 L |
| 15 | SOIL | D20COMP1 | 1 | | 2019/11/18 13:38 | 2.09 L |
| 16 | SOIL | D20COMP2 | 1 | | 2019/11/18 13:48 | 2.13 L |
| 17 | SOIL | D20COMP3 | 1 | | 2019/11/18 13:58 | 2.05 L |
| 18 | SOIL | D40NPK1 | 1 | | 2019/11/18 14:07 | 1.19 L |
| 19 | SOIL | D40NPK2 | 1 | | 2019/11/18 14:17 | 0.715 L |
| 20 | SOIL | D40NPK3 | 1 | | 2019/11/18 14:27 | 1.17 L |
| 21 | SOIL | D40COMP1 | 1 | | 2019/11/18 14:37 | 1.15 L |
| 22 | SOIL | D40COMP2 | 1 | | 2019/11/18 14:46 | 1.15 L |
| 23 | SOIL | D40COMP3 | 1 | | 2019/11/18 14:56 | 1.35 L |
| 24 | SOIL | D60NPK1 | 1 | | 2019/11/18 15:06 | 1.17 L |
| 25 | SOIL | D60NPK2 | 1 | | 2019/11/18 15:15 | 1.23 L |
| 26 | SOIL | D60NPK3 | 1 | | 2019/11/18 15:25 | 1.30 L |
| 27 | SOIL | D60HG1 | 1 | | 2019/11/18 15:35 | 0.582 L |
| 28 | SOIL | D60HG2 | 1 | | 2019/11/18 15:44 | 0.586 L |
| 29 | SOIL | D60HG3 | 1 | | 2019/11/18 15:54 | 0.578 L |
| 30 | SOIL | D60COMP1 | 1 | | 2019/11/18 16:04 | 0.743 L |
| 31 | SOIL | D60COMP2 | 1 | | 2019/11/18 16:13 | 0.876 L |
| 32 | SOIL | D60COMP3 | 1 | | 2019/11/18 16:23 | 0.756 L |
| 33 | PLANT | D20NPKSH | 1 | | 2019/11/18 16:33 | 0.575 L |
| 34 | PLANT | D20NPKRO | 1 | | 2019/11/18 16:42 | 0.367 L |
| 35 | PLANT | D20COMPS | 1 | | 2019/11/19 09:18 | 0.485 L |
| 36 | PLANT | D20COMPF | 1 | | 2019/11/19 09:28 | 0.514 L |
| 37 | PLANT | D20HGROC | 1 | | 2019/11/19 09:38 | 0.517 L |
| 38 | PLANT | D20NPKSH | 1 | | 2019/11/19 09:45 | 0.571 L |
| 39 | PLANT | D20NPKRO | 1 | | 2019/11/19 09:52 | 0.596 L |
| 40 | PLANT | D20COMPS | 1 | | 2019/11/19 09:59 | 0.471 L |
| 41 | PLANT | D20COMPF | 1 | | 2019/11/19 10:06 | 0.502 L |
| 42 | PLANT | D20HGSHC | 1 | | 2019/11/19 10:14 | 0.575 L |
| 43 | PLANT | D20NPKSH | 1 | | 2019/11/19 10:21 | 0.597 L |
| 44 | PLANT | D20NPKRO | 1 | | 2019/11/19 10:28 | 0.498 L |
| 45 | PLANT | D20COMPS | 1 | | 2019/11/19 10:35 | 0.532 L |
| 46 | PLANT | D20COMPF | 1 | | 2019/11/19 10:42 | 0.506 L |
| 47 | PLANT | D40HGSHC | 1 | | 2019/11/19 10:49 | 0.589 L |
| 48 | PLANT | D40HGROC | 1 | | 2019/11/19 10:57 | 0.554 L |
| 49 | PLANT | D40COMPF | 1 | | 2019/11/19 11:04 | 0.562 L |
| 50 | PLANT | D40COMPS | 1 | | 2019/11/19 11:11 | 0.564 L |
| 51 | PLANT | D20HGSHC | 1 | | 2019/11/19 11:18 | 0.601 L |
| 52 | PLANT | D20HGSHC | 1 | | 2019/11/19 11:25 | 0.362 L |

| | | | | | |
|---|---|---|---|---|---|
| 53 | SOIL | D40HG1 | 1 | 2019/11/19 11:33 | 0.642 L |
| 54 | SOIL | D40HG2 | 1 | 2019/11/19 11:40 | 0.653 L |
| 55 | SOIL | D40HG3 | 1 | 2019/11/19 11:47 | 0.821 L |
| 64 | PLANT | D40NPKRO | 1 | 2019/11/19 14:26 | 0,257 L |
| 65 | PLANT | D40NPKRO | 1 | 2019/11/19 14:31 | 0.184 L |
| 66 | PLANT | D40NPKRO | 1 | 2019/11/19 14:36 | 0.202 L |
| 67 | PLANT | D40NPKSH | 1 | 2019/11/19 14:40 | 0.121 L |
| 68 | PLANT | D40NPKSH | 1 | 2019/11/19 14:45 | 0.269 L |
| 69 | PLANT | D40NPKSH | 1 | 2019/11/19 14:50 | 0.280 L |
| 70 | PLANT | D40NPKSH | 1 | 2019/11/19 14:54 | 0.284 L |
| 71 | PLANT | D40NPKSH | 1 | 2019/11/19 14:59 | 0.315 L |
| 72 | PLANT | D40NPKSH | 1 | 2019/11/19 15:04 | 0.307 L |
| 73 | PLANT | D40NPKSH | 1 | 2019/11/19 15:08 | 0.308 L |
| 74 | PLANT | D40HGROC | 1 | 2019/11/19 15:13 | 0.293 L |
| 75 | PLANT | D40HGROC | 1 | 2019/11/19 15:18 | 0.291 L |
| 76 | PLANT | D40HGROC | 1 | 2019/11/19 15:22 | 0.288 L |
| 77 | PLANT | D40HGSHC | 1 | 2019/11/19 15:27 | 0.335 L |
| 78 | PLANT | D40HGSHC | 1 | 2019/11/19 15:32 | 0.340 L |
| 79 | PLANT | D40HGSHC | 1 | 2019/11/19 15:36 | 0.344 L |
| 80 | PLANT | D20HGROC | 1 | 2019/11/19 15:41 | 0.516 L |
| 81 | PLANT | D20HGROC | 1 | 2019/11/19 15:46 | 0.512 L |
| 82 | PLANT | D20HGROC | 1 | 2019/11/19 15:50 | 0.509 L |
| 83 | PLANT | D20HGROC | 1 | 2019/11/19 15:55 | 0.508 L |
| 84 | PLANT | D60NPKRO | 1 | 2019/11/19 16:00 | 0.686 L |
| 85 | PLANT | D60NPKRO | 1 | 2019/11/19 16:05 | 0.673 L |
| 86 | PLANT | D60NPKRO | 1 | 2019/11/19 16:09 | 0.0682 L |
| 87 | PLANT | D60NPKRO | 1 | 2019/11/19 16:14 | 0.0741 L |
| 88 | PLANT | D60NPKRO | 1 | 2019/11/19 16:19 | 0.0845 L |
| 89 | PLANT | D60NPKRO | 1 | 2019/11/19 16:23 | 0.546 L |
| 90 | PLANT | D60NPKRO | 1 | 2019/11/19 16:28 | 0.549 L |
| 91 | PLANT | D60NPKRO | 1 | 2019/11/19 16:33 | 0.580 L |
| 92 | PLANT | D60NPKSH | 1 | 2019/11/19 16:37 | 0.107 L |
| 93 | PLANT | D60NPKSH | 1 | 2019/11/19 16:42 | 0.735 L |
| 94 | PLANT | D60NPKSH | 1 | 2019/11/19 16:47 | 0.121 L |
| 95 | PLANT | D60NPKSH | 1 | 2019/11/19 16:51 | 0.168 L |
| 96 | PLANT | D60NPKSH | 1 | 2019/11/19 16:56 | 0.462 L |
| 97 | PLANT | D60NPKSH | 1 | 2019/11/19 17:01 | 0.225 L |
| 98 | PLANT | D60NPKSH | 1 | 2019/11/19 17:05 | 0.401 L |
| 99 | PLANT | D60NPKSH | 1 | 2019/11/19 17:10 | 0.0892 L |
| 100 | PLANT | D60NPKSH | 1 | 2019/11/19 17:15 | 0.175 L |
| 101 | PLANT | D60NPKSH | 1 | 2019/11/19 17:19 | 0.189 L |
| 102 | PLANT | D60COMPS | 1 | 2019/11/19 17:24 | 0.532 L |
| 103 | PLANT | D60COMPS | 1 | 2019/11/19 17:29 | 0.529 L |
| 104 | PLANT | D60COMPS | 1 | 2019/11/19 17:34 | 0.530 L |
| 105 | PLANT | D60COMPF | 1 | 2019/11/19 17:38 | 0.494 L |
| 106 | PLANT | D60COMPF | 1 | 2019/11/19 17:43 | 0.546 L |
| 107 | PLANT | D60COMPF | 1 | 2019/11/19 17:48 | 0.548 L |

| | | | | | | |
|---|---|---|---|---|---|---|
| 108 | PLANT | D60HGROC | 1 | 2019/11/19 | 17:52 | 0.523 L |
| 109 | PLANT | D60HGROC | 1 | 2019/11/19 | 17:57 | 0.524 L |
| 110 | PLANT | D60HGROC | 1 | 2019/11/19 | 18:02 | 0.544 L |
| 111 | PLANT | D60HGSHC | 1 | 2019/11/19 | 18:06 | 0.493 L |
| 112 | PLANT | D60HGSHC | 1 | 2019/11/19 | 18:11 | 0.501 L |
| 113 | PLANT | D60HGSHC | 1 | 2019/11/19 | 18:16 | 0.524 L |
| 114 | PLANT | D20CTRLRC | 1 | 2019/11/20 | 10:50 | -0.519 L |
| 115 | PLANT | D20CTRLRC | 1 | 2019/11/20 | 10:55 | -0.526 L |
| 116 | PLANT | D20CTRLRC | 1 | 2019/11/20 | 11:00 | -0.533 L |
| 117 | PLANT | D20CTRLSH | 1 | 2019/11/20 | 11:04 | -0.544 L |
| 118 | PLANT | D20CTRLSH | 1 | 2019/11/20 | 11:09 | -0.626 L |
| 119 | PLANT | D20CTRLSH | 1 | 2019/11/20 | 11:14 | -0.618 L |
| 120 | PLANT | D40CTRLRC | 1 | 2019/11/20 | 11:18 | -0.620 L |
| 121 | PLANT | D40CTRLRC | 1 | 2019/11/20 | 11:23 | -0.616 L |
| 122 | PLANT | D40CTRLRC | 1 | 2019/11/20 | 11:28 | -0.405 L |
| 123 | PLANT | D40CTRLSH | 1 | 2019/11/20 | 11:32 | -0.402 L |
| 124 | PLANT | D40CTRLSH | 1 | 2019/11/20 | 11:37 | -0.375 L |
| 125 | PLANT | D40CTRLSH | 1 | 2019/11/20 | 11:42 | -0.561 L |
| 126 | PLANT | D60CTRLRC | 1 | 2019/11/20 | 11:46 | -0.546 L |
| 127 | PLANT | D60CTRLRC | 1 | 2019/11/20 | 11:51 | -0.509 L |
| 128 | PLANT | D60CTRLRC | 1 | 2019/11/20 | 11:56 | -0.507 L |
| 129 | PLANT | D60CTRLSH | 1 | 2019/11/20 | 12:01 | -0.375 L |
| 130 | PLANT | D60CTRLSH | 1 | 2019/11/20 | 12:06 | -0.402 L |
| 131 | PLANT | D60CTRLSH | 1 | 2019/11/20 | 12:14 | -0.405 L |

## Appendix C: Calculations of Total percentage of Hg uptake by sweet sorghum in Hg-contaminated soil

The calculations were done using the below equation (Titah *et al.*, 2013)

$$\text{Total Hg uptake (\%)} = \frac{\text{CHg in plant x DW plant x N plant}}{\text{C Bioavailable Hg in soil x W soil}} \times 100$$

where,

CHg in plant = concentration of Hg uptake per plant (mg $kg^{-1}$)

DW plant = dry weight of plant (Kg)

N plant = number of plants

C bioavailable Hg = concentration of Hg bioavailable in soil (mg $kg^{-1}$)

W soil = total weight of soil (Kg)

**Total percentage of Hg uptake after Day 20 of exposure of sweet sorghum in Hg-contaminated soil:**

**Hg only (%):** $\frac{0.55 \times 0.16 \times 5}{1.79 \times 2.5} \times 100$ **= 9.83%**

**Hg + Compost (%):** $\frac{0.51 \times 0.21 \times 5}{2.09 \times 2.5} \times 100$ **= 10.25%**

**Hg + NPK (%):** $\frac{0.58 \times 0.16 \times 5}{1.76 \times 2.5} \times 100$ **= 10.55%**

**Total percentage of Hg uptake after Day 40 of exposure of sweet sorghum in Hg-contaminated soil:**

**Hg only (%):** $\frac{0.49 \times 0.11 \times 5}{1.03 \times 2.5} \times 100$ **= 10.47%**

**Hg + Compost (%):** $\frac{0.59 \times 0.25 \times 5}{1.21 \times 2.5} \times 100$ **= 24.38%**

**Hg + NPK (%):** $\frac{0.31 \times 0.87 \times 5}{1.03 \times 2.5} \times 100$ **= 52.37%**

**Total percentage of Hg uptake after Day 60 of exposure of sweet sorghum in Hg-contaminated soil:**

**Hg only (%):** $\frac{0.53 \times 0.20 \times 5}{0.90 \times 2.5} \times 100$ **= 23.56%**

**Hg + Compost (%):** $\frac{0.58 \times 0.18 \times 5}{0.79 \times 2.5} \times 100$ **= 26.43%**

**Hg + NPK (%):** $\frac{0.61 \times 0.85 \times 5}{1.23 \times 2.5} \times 100$ **= 84.31%**

## REFERENCES


Abou-Shanab, R.A., Angle, J.S., Delorme, T.A., Chaney, R.L., van Berkum, P., Moawad, H., Ghanem, K., Ghozlan, H.A. (2003). Rhizobacterial effects on nickel extraction from soil and uptake by Alyssum murale. *New Phytol*. 158 (1), 219–224.

Ahammad, S.J., Sumithra, S., Senthilkumar, P., (2018). Mercury uptake and translocation by indigenous plants. *Rasayan J. Chem.* 11, 1–12.

A.O.A.C. (1980). *Official Methods of Analysis*, 13th ed. Association of Official Analytical Chemists. Washington D.C. p 36-39.

Al-Chami, Z., Amer, N., Al Bitar, L., Cavoski, I. (2015). Potential use of *Sorghum bicolor* and *Carthamus tinctorius* in phytoremediation of nickel, lead, and zinc. *Int. J. Environ. Sci. Technol* 12, 3957–3970.

Ali, H., Khan E., Sajad, M.A. (2013). Phytoremediation of heavy metals—Concepts and applications. *Chemosphere.* 91, 869–881.

Ashraf, M.A., Jamil, M.M., Yusoff, I. (2013). Evaluation of Natural Phytoremediation Process Occurring at Ex-tin Mining Catchment. *Chiang Mai J. Sci.* 2013; 40(2): 198-213.

Assad, M., Parelle, J., Cazaux, D., Gimbert, F., Chalot, M., Tatin-Froux, F. (2016). Mercury uptake into poplar leaves. *Chemosphere.* 146, 1–7.

ATSDR. (2009). Toxicological profile for mercury. Atlanta, GA; U.S. Department of Health and Human Services, Public Health Service.

Baker, A.J.M., and Brooks, R.R., (1989). Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. *Bio-recovery.* 1, 81–126.

Barker, A.J.M., McGrath, S.P., Sidoli, C.M.D., Reeves, R.D. (1994). The possibility of in situ heavy metal decontamination of polluted soil using crops of metal-accumulating plants. *Resour. Conserv. Recy.* 11: 41-49.

Barman, S.C., Sahu, R.K., Bhargava, S.K., Chaterjee, C. (2000). Distribution of heavy metals in wheat, mustard, and weed grown in field irrigated with industrial effluents. *Bulletin of Environmental Contamination and Toxicology.* 64: 489–496.

Brunetti, G., Farrag, K., Rovira, P.S., Nigro, F., Senesi, N. (2011). Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by Brassica napus from contaminated soils in the Apulia region, Southern Italy. *Geoderma*. 160 (2011) 517–523.

Cao, X. and Ma, L.Q. (2004). Effects of compost and phosphate on plant arsenic accumulation from soils near pressure-treated wood. *Environmental Pollutant.* 132, 435–442.

Chang, T. and Yen, J. (2006). On-site mercury-contaminated soils remediation by using thermal desorption technology, *J. Hazard. Mater.* 128 (2006) 208–217.

Cheng, J., Yuan, T., Wang, W., Jia, J., Lin, X., Qu, L., Ding, Z. (2006). Mercury pollution in two typical areas in Guizhou province, China and its neurotoxic effects in the brains of rats fed with local polluted rice, *Environ. Geochem. Health* 28, 499–507.

Conley, T., Hulet, G., Morris, M., Osbone-Lee, I. (1999). *Demonstration Results on the Effects of Mercury Speciation on the Stabilization of Wastes*, ORNL/TM- 1999/120, Oak Ridge National Laboratory, Oak Ridge, TN, 1999.

Cox, C.D., Shoesmith, M.A., Ghosh, M.M. (1996). Electrokinetic remediation of mercury contaminated soils using iodine/iodide lixiviant, *Environ. Sci. Technology* 30, 1933- 1938.

Cynthia, R.E. and David, A.D. (1997). *Remediation of Metals-Contaminated Soils and Groundwater-Technology Evaluation* Report E Series: TE-97-01, Carnegie Mellon University, Department of Civil and Environmental Engineering Pittsburgh, PA, 1997.

Dastoor, A.P. and Larocque, Y. (2004). (Global circulation of atmospheric mercury: a modeling study, *Atmos. Environ.* 38, 147–161.

Department of Environmental Affairs (DEA, 2012). National Environmental Management: Waste Act (59/2008): Draft national norms and standards for the remediation of contaminated land and soil quality: Republic of South Africa Government Gazette. Vol 561, No. 35160. Government Printing works, Pretoria.

Dermont, G., Bergeron, M., Mercier, G., Richer-Lafleche, M. (2008). Metal-contaminated soils: remediation practices and treatment technologies. *Pract. Period. Hazard. Tox. Radioact. Waste Manage* 12, 188–210.

Du Plessis, J. (2008). Sorghum Production. Department of Agriculture in cooperation with the ARC-Grain Crops Institute, Pretoria: Department of Agriculture, RSA.

Ermolin, M.S., Fedotov, P.S., Malik, N.A., Karandashev, V.K. (2018). Nanoparticles of Volcanic Ash as a Carrier for Toxic Elements on the Global Scale. *Environment International* 200, 22.

Esalah, O.J., Weber, M.E., Vera, J.H. (2000). Removal of lead, cadmium and zinc from aqeous solutions by precipitation with sodium di (n-octyl) phosphinate. *Canad. Journ. of Chem. Eng.* 78 (5), 948-954.

Feng, X., Dai, Q., Qiu, G., Li, L. He, D., Wang, G. (2006). Gold mining related mercury contamination in Tongguan, Shaanxi Province, PR China, *Appl. Geochem.* 21, 1955–1968.

Feng, X., Li, P., Qiu, G., Wang, S., Li, G., Shang, L., Meng, B., Jiang, H., Bai, W., Li, Z. (2007). Human exposure to methylmercury through rice intake in mercury mining areas, Guizhou Province, China, *Environ. Sci. Technol.* 42, 326–332.

Fish, L. & Victor, J.E. (2005). *Sorghum bicolor* (L.) Moench subsp. arundinaceum (Desv.) de Wet & Harlan. National Assessment. Pretoria: Red List of South African Plants version 2017.1.

Frost, J. (2020). Hypothesis testing: An intuitive guide for making Data driven decisions. statisticsbyjim.com

Gonzaga, M.I.S., Santos, J.A.G., Ma, L.Q. (2006). Arsenic phytoextraction dan hyperaccumulation by fern species. *Sci. Agric.* (*Piracicaba, Braz.*). 63 (1), 90–101.

Gulz, P.A. (2002). *Arsenic uptake of common crop plants from contaminated soils and interaction with phosphate*. Dissertation from the Swiss Federal Institute of Technology Zurich for the Degree of Doctor of Natural Science, Switzerland.

Gupta, D.K., Chatterjee, S., Datta, S., Veer, V., Walther, C. (2014). Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals. *Chemosphere*. 108 (2014) 134–144.

Han, F.X., Shiyab, S., Chen, J., Su, Y., Monts, D.L., Waggoner, C.A., Matta, F.B. (2008). Extractability and bioavailability of mercury from a mercury sulfide contaminated soil in Oak Ridge, Tennessee, USA, *Water Air Soil Pollut.* 194.

Handreck, K.A. and Black, N.D. (1994). *Growing Media for ornamental plants and turf.* Revised (Revised Ed.) Univ. of New South Wales Press.

He, F., Gao, J., Pierce, E., Strong, PJ., Wang, H., Liang, L. (2015). In situ remediation technologies for mercury-contaminated soil. *Environ Sci Pollut Res.*22, 8124–8147.

Hidayati, N., Juhaeti, T., Syarif, F. (2009). Mercury and cyanide contaminations in Gold Mine Environment and Possible Solution of Cleaning up by using phytoextraction. *Journal of Biosciences* 16, 88-94.

Hignett, T.P. (1991). *Chemical Analysis of Fertiliser Materials*: Recommendations of the Fertiliser Liaison Committee. Springer Science.

Huang, Y.T., Hseu, Z.Y., Hsi, H.C. (2011). Influences of thermal decontamination on mercury removal, soil properties, and repartitioning of coexisting heavy metals, *Chemosphere* 84, 1244–1249.

Ibrahim, S.I., Abdel Lateef, M.F., Khalifa, H.M.S., Abdel Monem, A.E. (2013). Phytoremediation of atrazine-contaminated soil using *Zea mays* (maize). *Annals of Agricultural Science* (2013) 58(1), 69–75.

Ijaz, A., Imran, A., ul Haq, M.A., Khan, Q.M., Afzal, M. (2016). Phytoremediation: recent advances in plant-endophytic synergistic interactions. *Plant Soil*, 405 (1–2), 179–195.

ITRC (1997). Emerging technologies for the remediation of metals in soils: phytoremediation. Interstate Technology and Regulatory Cooperation Work Group, http://www.itrcweb.org/Documents/ MIS-5.pdf.

ITRC (1998). Technical Guidelines for On-Site Thermal Desorption of Solid Media and Low-Level Mixed Waste Contaminated with Mercury and/or Hazardous Chlorinated Organics Final Report, ITRC Work Team of Low Temperature Thermal Desorption, Washington, DC, 1998.

Jimenez, R.R. & Ladha, J.K. (1993). "Automated elemental analysis: A rapid and reliable but expensive measurement of total carbon and nitrogen in plant and soil samples". *Commun. Soil Sci. Plant Anal.* 24, 1897-1924.

Khan, M.S., Zaidi, A., Wani, P.A., Oves, M. (2009). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils: a review. In: Lichtfouse, E. (Ed.), Organic Farming, Pest Control and Remediation of Soil Pollutants, Sustainable Agriculture Reviews 1. *Springer Science and Business Media B.V., pp.* 319–350.

Kokyo, O.H., Takahi, S., Wedhastri, S., Sudarmawan, L.H., Rosariastuti, R., Prijambada, I.D. (2015). Phytoremediation of mercury contaminated soils in a small-scale artisanal gold mining region of Indonesia. *International Journal of Bioscience and Biotechology*. Vol. II, 15-21.

Kot, F.S., Rapoport, V.L., Kharitonova, G.V. (2007). Immobilization of soil mercury by colloidal sulphur in the laboratory experiment, *Cent. Eur. J Chem. 5*, 846–857.

Kucharski, R., Zielonka, U., Sas-Nowosielska, A., Kuperberg, J.M., Worsztynowicz, A., Szdzuj, J. (2005). A method of mercury removal from topsoil using low-thermal application. *Environ. Monit. Assess.* 104, 341–351.

Kumar, B., Smita, K., Flores, LC. (2014). Plant mediated detoxification of mercury and lead. *Arabia Journal of Chemistry* 12, 300-318.

Leedy, P.D. and Ormrod, J.E. (2015). *Practical Research:* Planning and Design (11th Ed). Global Edition. Boston: Pearson.

Lehmann, J. and Kleber, M. (2015). The contentious nature of soil organic matter. *Nature* 528, 60-81.

Li, H., Shi, W., Shao, H., Shao, M. (2009). The remediation of the lead-polluted garden soil by exogenous natural zeolite. *J. Hazard. Mater.* 169 (1-3), 1106-1111.

Luo, S., Xu, T., Chen, L., Chen, J., Rao, C., Xian, X., Wan, Y., Zeng, G., Long, F., Liu, C., Liu, Y. (2012). Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte *Bacillus* sp. SLS18. *Appl Microbiol Biotechnol*. 93, 1745-1753.

Malar, S., Sahi, S.V., Favas, P.J., Venkatachalam, P. (2015). Mercury heavy metal induced physiochemical changes and genotoxic alterations in water hyacinths. Eichhornia crassipes (Mart.). *Environ. Sci. Pollut. Res*. 22, 4597–4608.

Malehase, T., Adegbenro, P.D., Okonkwo, J.O. (2016). Determination of mercury and its fractionation products in samples from legacy use of mercury amalgam in gold processing in Randfontein, South Africa. *Emerging Contaminants* 2, 157-165.

Marrugo-Negrete, J., Durango-Hernanadez, J., Pinedo-Hernandez, J., Olivero-Verbel, J., Diez, S. (2015). Phytoremediation of mercury-contaminated soil by *Jatropha curcas*. *Chemosphere* 127, 58-63.

Mason, R.P., Fitzgerald, W.F., Morel, F.M.M. (1994). The biogeochemical cycling of elemental mercury - anthropogenic influences. *Geochim. Cosmochim. Acta* 58, 3191–3198.

Massacci, P., Piga, L., Ferrini, M. (2000). Applications of physical and thermal treatment for the removal of mercury from contaminated materials, *Miner. Eng.* 13, 963–967.

Meng, D.K., Chen, J., Yang, Z.M. (2011). Enhancement of tolerance of Indian mustard (*Brassica juncea*) to mercury by carbon monoxide. *J. Hazard. Mater*. 186, 1823–1829.

Mouton, J. (2014). *How to succeed in your Master's & Doctoral Studies:* A South African Guide and Resource Book. (20th Ed). Pretoria: Van Schaik.

Mulligan, C.N., Yong, R.N., Gibbs, B.F. (2001). An evaluation of technologies for the heavy metal remediation of dredged sediments, *J. Hazard. Mater.* 85, 145–163.

Nagajyoti, P., Lee, K., Sreekanth, T. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environ. Chem. Lett.* 8, 199–216.

Natasha, M.S., Khalid, S., Bibi, I., Bundschuh, J., Niazi, N.K., Dumat, C. (2020). A critical review of mercury speciation, bioavailability, toxicity and detoxification in soil-plant environment: Ecotoxicology and health risk assessment. *Science of the Total Environment*. 711, 134-749.

Navarro, A., Caadas, I., Martinez, D., Rodriguez, J., Mendoza, J. (2009). Application of solar thermal desorption to remediation of mercury-contaminated soils. *Sol. Energy* 83, 1405–1414.

NCBI. National Center for Biotechnology Information. (2018). PubChem Compound Database; CID=23931, https://pubchem.ncbi.nlm.nih.gov/compound/23931 (accessed Aug. 18, 2018).
toxicity to alfalfa (Medicago sativa) plants. *Chemosphere* 70, 1500–1509.

Nicolardi, V., Cai, G., Parrotta, L., Puglia, M., Bianchi, L., Bini, L. (2012). The adaptive response of lichens to mercury exposure involves changes in the photosynthetic machinery. *Environ. Pollut.* 160, 1–10.

Oramah, I.T., Richards, J.P., Summers, R., Garvin, T. (2015). Artisanal and small-scale mining in Nigeria: Experiences from Niger, Nasarawa and Plateau states. *The Extractive Industries and Society*. 2 (2015) 694 – 704.

Pataranawat, P., Parkpian, P., Polprasert, C., Delaune, R., Jugsujinda, A. (2007). Mercury emission and distribution: Potential environmental risks at a small-scale gold mining operation, Phichit Province, Thailand, *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.* 42, 1081–1093.

Perez-Sanz, A., Millan, R., Sierra, M.J., Alarcon, R., Garcia, P., Gil-Diaz, M., Vazquez, S., Carmen-Lobo, M. (2012). Mercury uptake by *Silene vulgaris* grown on contaminated spiked soils. *J Environ Manag* 95: 5233-5237.

Piao, H. and Bishop, P.L. (2006). Stabilization of mercury-containing wastes using sulphide, *Environ. Pollut.* 139, 498–506.

Pilon, S.E. (2000). Phytoremediation, *Ann. Rev. Plant Biol*., 2005; 56: 15-39.

Quevauviller, P.H. (1998). *Methodologies in Soil and Sediment Fractionation Studies, Single and Sequential Extraction Procedures.* Royal Society of Chemistry, UK.

Randall, P.M. and Chattopadhyay, S. (2010). Bench-scale evaluation of chemically bonded phosphate ceramic technology to stabilize mercury waste mixtures, *J. Environ. Eng.* 136, 265–273.

Rascio, N., Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant Sci.* 180 (2), 169–181.

Reddy, K.R., Chaparro, C., Saichek, R.E. (2003). Removal of mercury from clayey soils using electrokinetics, *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ.* Eng. 38, 307–338.

Rieser, L.A., Bishop, P., Suidan, M.T., Piao, H., Fauche, R.A., Zhang, J., Randall, P. (2001). *Stabilization and Testing of Mercury Containing Wastes: Borden Catalyst EPA/600/R-02/019,* U.S

EPA National Risk Management Research Laboratory/Office of Research and Development, Cincinnati/Ohio, 2001.

Salt, D.E., Smith, R.D., Raskin, I. (1998). Phytoremediation. *Annu. Rev. Plant Phys*. 49, 643–668.

Sarwar, N., Imaran, M., Shaheen, M.R., Ishaque, W., Kamran, M.A., MAtloob, A., Rehim, A, and Hussain, S. (2017). Phytoremediation strategies for soil contaminated with heavy metals: Modifications and future perspectives. *Chemosphere*, 17, 710-721.

Sathya, A., Kanaganahalli, V., Srinivas Rao, P., Gopalakrishnan, S. (2016). Cultivation of Sweet Sorghum on Heavy Metal Contaminated Soils by Phytoremediation Approach for Production of bioethanol. *Bioremediation and Bioeconomy* 2016, 271–292.

Schroeder, W.H. and Munthe, J. (1998). Atmospheric mercury–an overview, *Atmos. Environ.* 32, 809–822.

Semu, E., Singh, B.R., Selmer-Olsen, A.R. (1987). Adsorption of mercury compounds by tropical soils II. Effect of soil: solution ratio, ionic strength, pH, and organic matter, *Water, Air, Soil Pollut* 32, 1–10.

Shao, H.B., Chu, L.Y., Ruan, C.J., Li, H., Guo, D.G., Li, W.X. (2010). Understanding molecular mechanism for improving phytoremediation of heavy metal contaminated soil. *Crit. Rev. Biotech.* 30 (1), 23-30.

Shen, Z., Zhang, J., Qu, L., Dong, Z., Zheng, S., Wang, W. (2009). A modified EK method with an I-/I2 lixiviant assisted and approaching cathodes to remedy mercury contaminated field soils, *Environ. Geol*. 57, 1399–1407.

Sierra, C., Gallego, J., Afif, E., Menendez-Aguado, J., Gonzalez-Coto, F. (2010). Analysis of soil washing effectiveness to remediate a brownfield polluted with pyrite ashes, *J. Hazard. Mater.* 180, 602–608.

Smolinska, B. (2015). Green waste compost as an amendment during induced phytoextraction of mercury-contaminated soil. *Environ Sc Pollut Res* (2015) 22: 3528-3537.

Telmer, K., and Stapper, D.A. (2012). *Practical Guide: Reducing Mercury Use in Artisanal and Small-scale Gold Mining.* Nairobi, Kenya and Geneva, Switzerland: United Nations Environment Programme.

Thomas, J.M., and Ward, C.H. (1993). *Introduced organisms for subsurface bioremediation.* pp. 227-244. In Norris et al. (Ed). Handbook of bioremediation. Florida: Lewis Publ., Boca Raton.

Titah, H.S., Sheikh Abdullaha, S.R., Mushrifahc, I., Anuara, N., Basrib, H., Mukhlisinb, M. (2013). Effect of applying rhizobacteria and fertilizer on the growth of Ludwigia octovalvis for arsenic uptake and accumulation in Phytoremediation. *Ecological Engineering* 58, 303– 313.

Trüe, A., Panichev, N., Okonkwo, J., Forbes, P.B.C. (2012). Determination of mercury content of Lichens and comparison to atmospheric mercury level in South African Highveld region, *Clean Air J. 21*, 19-25.

Truex, M.J., Oostrom, M., Zhang, Z.F., Carroll, K.C., Schramke, J.A., Wietsma, T.W., Tartakovsky, G.D., Gordon, K.A., and Last, G.V. (2010). Evaluation of soil flushing for application to the deep vadose zone in the Hanford Central Plateau. *Pacific Northwest National Laboratory Report PNNL*-19938.

Tu, C. and Ma, L.Q. (2003). Effects of arsenate and phosphate on their accumulation by an arsenic-hyperaccumulator Pteris vittata L. *Plant Soil.* 249, 373–382.

Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A. (2001). Mercury in the aquatic environment: a review of factors affecting methylation, Crit. Rev. *Env. Sci. Technol.* 31, 241–293.

UNEP. (2013). *Mercury Artisanal and Small-Scale Gold Mining (ASGM) Partnership Area*. Geneva: United Nations Environment Programme.

U.S. Environmental Protection Agency. (1996). *Environmental Protection Agency Volunteer Monitor's Guide to: Quality Assurance Project Plans.* Washington, DC: National Centre for Environmental Assessment, Office of Research and Development.

U.S. Environmental Protection Agency. (1997a). *Treatment Technologies for Mercury in Soil, Waste and Water,* Washington, DC, EPA-542-R-07-003, 1997.

U. S. Environmental protection Agency. (1997b). *Technology Alternatives for the Remediation of Soils Contaminated with As, Cd, Cr, Hg, and Pb* EPA/540/S-97/500, Cincinnati, OH: US. EPA Office of Research and Development.

U. S. Environmental protection Agency. (2000). *Introduction to phytoremediation.* U.S. EPA Report EPA/600/R-99/107.

U. S. Environmental protection Agency. (2002). Arsenic Treatment Technology for Soil, Waste and Water. www.epa.gov/tioclu-in.org/arsenic.

U.S. Environmental Protection Agency. (2007). *Treatment Technologies for Mercury in Soil, Waste, and Water.* Washington, DC: U.S. Environmental Protection Agency, Office of Superfund Remediation and Technology Innovation.

U.S. Environmental Protection Agency. (2008). *Integrated Risk Information System (IRIS) on Arsenic*. Washington, DC: National Centre for Environmental Assessment, Office of Research and Development.

Utami, D., Takahi, S., Prijambada, D. I. (2016). *Mercury Accumulation in Gold Mine Tailing by Sweet Sorghum Inoculated with Chromium Uptake Enhancing Rhizobacteria*. Udayana: Udayana University. Bioscience and Biotechnology forum.

Virkutyte, J., Sillanp, M., Latostenmaa, P. (2002). Electrokinetic soil remediation—critical overview, *Sci. Total Environ.* 289, 97–121.

Walker, C.H., Sibly, R.M., Hopkin, S.P., and Peakall, D.B. (2014). *Principles of Ecotoxicology*. 4th ed. CRC Press. New York: Taylor & Francis Group.

Wang, J., Feng, X., Anderson, W.C.N., Xing, Y., Shang, L. (2012). Remediation of mercury contaminated sites – A review. *Journal of Hazardous Materials 2*21– 222, 1– 18.

Wolfe, M.F., Schwarzbach, S., and Sulaiman, R.A. (1998). Comprehensive review of distribution and effects of mercury in the environment. *Environmental Toxicology and Chemistry* 17, 146-160.

World Health Organization. (2019). Mercury and health. World Health Organization. Accessed on September 16, 2019: https://www.who.int/news-room/fact-sheets/detail/ mercury-and-health.

Xia, H., Wu, L. and Tao Q., (2003). A review on phytoremediation of organic contaminants, *Chinese J. Applied Ecol.*, 14, 457-460.

Xing, B. & Yeneman, P.L.M. (1998). Microwave digestion for analysis of metals in soil, Communications in Soil Science and Plant Analysis, 29:7-8, 923-930.

Xiong, Z., He, F., Zhao, D., Barnett, M.O. (2009). Immobilization of mercury in sediment using stabilized iron sulfide nanoparticles, *Water Res.* 43, 5171–5179.

Yoon, J., Cao, X., Zhou, Q., Ma, L. (2006). Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.* 368, 456-464.

Zasoski, R.J. and Burau, R.G. (1977). A Rapid Nitric-Perchloric Acid Digestion Method for Multi Element Tissue Analysis. *Communications in Soil Science and Plant Analysis*, 8(5), 425-436.

Zekely, A., Poor, P., Bagi, I., Csiszar, J., Gemes, K., Horvath, F., Tari, I. (2011). Effect of EDTA on the growth and copper accumulation of sweet sorghum and Sudan grass seedlings. *Acta Biol. Szeged*, 55, 159-164.

Zhang, T., Lu, Q., Su, C., Yang, Y., Hu, D., Xu, Q. (2017). Mercury induced oxidative stress, DNA damage, and activation of antioxidative system and Hsp70 induction in duckweed (*Lemna minor*). *Ecotoxicol. Environ. Saf.* 143, 46–56.

Zhou, Z.S., Wang, S.J., Yang, Z.M. (2008). Biological detection and analysis of mercury toxicity to alfalfa (*Medicago sativa*) plants, *Chemosphere*. 70, 1500–1509.

Zhuang, P and Shao J. (2009). Removal of metals by sorghum plants from contaminated land. *Journal of Environmental Sciences.* 21 (10), 1432-1437.

Zu, Y.Q., Li, Y., Chen, H.Y., Qin, L., Schvartz, C. (2005). Hyperaccumulation of Pb, Zn and Cd in herbaceous grown on lead-zinc mining area in Yunnan, China. *Environ Intl* 31, 755-762.

Zweiebel, F. (2005). *QA/QC for Environmental measurement, Module 13 Lecture Presentation,* Minnesota: University of Minnesota, USA.